

INDIAN COUNCIL OF MEDICAL RESEARCH
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SCIENTIFIC ADVISORY BOARD
FOR THE YEAR 1957

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OF THE
SCIENTIFIC ADVISORY BOARD
FOR THE YEAR
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Dr. V.S. Mangalik, Principal, K.G. Medical College, Lucknow.

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Dr. (Smt.) H.M. Sharma, Assistant Director of Public Health, 4, Kilpauk Garden Road, Madras-10.

Dr. C.R. Das Gupta, 1125, Prince Gulam Mohammad Road, Calcutta.

Shri K.K. Mathen, All-India Institute of Hygiene & Public Health, Calcutta.

Dr. J.N. Pohowalla, Reader in Pædiatrics, M.G.M. Medical College, Indore.

Dr. M.K. Krishna Menon, Director, Institute of Obstetrics and Gynæcology, Government Hospital for Women and Children, Madras-8.

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Dr. M.V. Radhakrishna Rao, Assistant Director, In-charge of the Department of Nutrition, Government of Bombay, Haffkine Institute, Parel, Bombay.

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Dr. V.N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor.

Dr. K. Mitra, 3, Shashi Ghosh Lane, Calcutta-5.

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Dr. Inderjit Singh, Professor of Physiology, Medical College, Agra.

Dr. J.D. Pathak, Professor of Physiology, Medical College, Baroda.

Dr. K.S. Sanjivi, Professor of Medicine & Physician, Madras Medical College and Government General Hospital, Madras.

Dr. G.K. Karandikar, Professor of Pharmacology, Medical College, Baroda. (Secretary)

Physiology of Human Reproduction

Dr. V.R. Khanolkar, Director, Indian Cancer Research Centre, Bombay. (Chairman)

Dr. B. Mukerji, Director, Central Drug Research Institute, Lucknow.

Dr. P.K. Malkani, Professor of Gynæcology and Obstetrics, Lady Hardinge Medical College, New Delhi.

Dr. V.N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor.

Dr. B.K. Anand, Professor of Physiology, All-India Institute of Medical Sciences, New Delhi.

Lieut.-Colonel B.L. Raina, Officer on Special Duty (Family Planning) Directorate-General of Health Services, New Delhi. (Secretary)

Virus Diseases

Dr. T.H. Work, Director, Virus Research Centre, Poona. (Chairman)

Dr. C.G.S. Iyer, Senior Research Officer, Neuropathological Unit, Indian Cancer Research Centre, Parel, Bombay-12.

Dr. I.G.K. Menon, Observer, Govt. of India Influenza Centre, Pasteur Institute of Southern India, Coonoor.

Dr. D.W. Soman, Director, Haffkine Institute, Bombay.

Shri M.R. Dhanda, Head of the Division of Pathology and Bacteriology, Indian Veterinary Research Institute, Izatnagar, (by nomination)

Lieut.-Colonel S.L. Kalra, Assistant Professor of Pathology, Armed Forces Medical College, Poona.

Dr. T. Ramchandra Rao, Assistant Director of Public Health (Malaria), Bombay State, Connaught House, Poona.

Dr. J.B. Shrivastav, Professor of Pathology & Bacteriology, Medical College, Nagpur.

Dr. N. Veeraraghavan, Director, Pasteur Institute of Southern India, Coonoor.

Dr. A.K. Thomas, Assistant Director, Centre Research Institute, Kasauli. (Secretary)

Committee for Developing Standard Methods of Analysis of Water, Sewage and Trade Effluents and Formulating Standards of Quality, etc.

Shri N.V. Modak, Special Engineer, Bombay Municipal Corporation, Mercantile Bank Building, Mahatma Gandhi Road, Bombay-1. (Chairman)

Dr. T.R. Bhaskaran, Associate Professor of Sanitary Engineering, All-India Institute of Hygiene & Public Health, 110, Chittaranjan, Avenue, Calcutta.

Shri L.P. Borkar, Superintendent and Chemist, Sewage Purification Works, Tulsi Pipe Road, Dadar, Bombay-28.

Dr. S. Govindarajan, Director, King Institute, Guindy, Madras-15.

Dr. M.I. Gurbaxani, Town Chemist, Tata Iron & Steel Co., Ltd., Jamshedpur.

Shri O.C. Hopkins, Ex-Adviser in Public Health Engineering, Directorate-General of Health Services, New Delhi. *(has since left India)*

Dr. Y.S. Narayana Rao, No. 5, II Crescent Park Road, Gandhi Nagar, Adyar, Madras-20.

Dr. S.C. Pillai, Department of Biochemistry, Indian Institute of Science, P.O. Malleswaram, Bangalore.

Shri K. Venkataramanan, Sanitary Chemist. (Public Health Engineering Organisation) Directorate-General of Health Services, New Delhi.

Dr. K.G. Veeraraghavan, Chief Water Analyst, King Institute, Guindy, Madras. *(Secretary)*

Cardio-vascular Diseases and Hypertension Sub-Committee

Dr. R.J. Vakil, Standard Building, 346 Hornby Road, Fort, Bombay. *(Chairman)*

Dr. B.K. Anand, Professor of Physiology, All-India Institute of Medical Sciences, New Delhi.

Dr. C. Gopalan, Deputy Director, Nutrition Research Laboratories, Coonoor.

Dr. R.P. Malhotra, Professor of Clinical Medicine, Medical College, Amritsar.

Dr. K.S. Sanjivi, Professor of Medicine and Physician, Madras Medical College and Government General Hospital, Madras.

Dr. A.K. Bose, 128-B, Dharmtalla Street, Calcutta-13.

Dr. G.G. Hadley, Associate Professor of Pathology, Christian Medical College, Vellore.

Dr. (Kumari) S. Padmavati, Professor of Medicine, Lady Hardinge Medical College, New Delhi.

Dr. P.K. Sen, Professor of Surgery, Seth G.S. Medical College, Bombay.

Dr. K.S. Mathur, Cardiologist, S.N. Medical College, Agra. *(Secretary)*

Hæmatological Sub-Committee

Dr. K.L. Wig, Principal, Medical College, Amritsar. *(Convener)*

Dr. J.B. Chatterjea, Professor of Hæmatology, School of Tropical Medicine, Calcutta.

Dr. N.M. Purandare, Professor of Pathology and Bacteriology, Seth G.S. Medical College, Bombay.

Dr. J.C. Patel, Honorary Assistant Physician, K.E.M. Hospital, Back Bay View, New Queen's Road, Bombay-4.

Dr. Sarad Kumar, Lecturer, Department of Pathology and Bacteriology, K.G. Medical College, Lucknow.

Liver Diseases Sub-Committee

- Dr. V.R. Khanolkar, Director, Indian Cancer Research Centre, Parel,
Bombay-12. (Chairman)
- Dr. S.T. Achar, Professor of Pædiatrics, Madras Medical College and
Government General Hospital, Madras.
- Dr. A.K. Basu, Director, Department of Surgery, Institute of Post-
Graduate Medical Education and Research, Calcutta-20.
- Dr. V.N. Patwardhan, Director, Nutrition Research Laboratories,
Coonoor.
- Dr. S.S. Sriramacharyulu, Pathologist, Nutrition Research Laboratories,
Coonoor.
- Dr. B.K. Aikat, Director, Department of Pathology and Bacteriology,
Seth Karnani Memorial Hospital, Lower Circular Road, Calcutta-20.
- Dr. R.M.L. Mehrotra, Reader in Pathology, K.G. Medical College,
Lucknow.
- Dr. M. Thangavelu, Professor of Pathology, Medical College, Trivan-
drum.
- Dr. P.N. Wahi, Professor of Pathology, S.N. Medical College, Agra.
- Dr. V. Ramalingaswami, Professor of Pathology, All-India Institute of
Medical Sciences, New Delhi. (Secretary)

Therapeutic Trials Sub-Committee

- Dr. B.B. Yodh, 18, Darabsha Road, Bombay. (Convener)
- Dr. R.N. Chaudhuri, Director, School of Tropical Medicine, Calcutta.
- Lieut.-Colonel Jaswant Singh, Deputy Director General of Health
Services, New Delhi.
- Dr. G.K. Karandikar, Professor of Pharmacology, Medical College,
Baroda.
- Dr. B. Mukerji, Director, Central Drug Research Institute, Lucknow.
- Dr. R.M. Kasliwal, Principal, S.M.S. Medical College, Jaipur.
- Dr. J.C. Patel, Honorary Assistant Physician, K.E.M. Hospital, Back Bay
View, New Queen's Road, Bombay-4.

Cholera Sub-Committee

- Dr. K.V. Venkatraman, Serologist and Chemical Examiner to the
Government of India, School of Tropical Medicine, Calcutta.
(Chairman)
- Dr. S.N. De, Professor of Pathology, Medical College, Calcutta.
- Shri K.K. Mathen, All-India Institute of Hygiene & Public Health,
Calcutta.
- Dr. S.C. Seal, Professor of Epidemiology, All-India Institute of Hygiene
& Public Health, Calcutta.

Dr. M.N. Lahiri, Professor of Microbiology, All-India Institute of Hygiene & Public Health, 110, Chittaranjan Avenue. Calcutta.

Dr. E.K. Narayanan, Assistant Director, Central Research Institute, Kasauli.

Dr. Gurkirpal Singh, Assistant Director, Central Research Institute, Kasauli. (Secretary)

Leprosy Sub-Committee

Dr. Dharmendra, Director, Central Leprosy Teaching & Research Institute, Tirumani (Chingleput Distt.) (Chairman)

Dr. Paul W. Brand, Professor of Orthopædics and Head of the Surgical Department, Christian Medical College, Vellore.

Dr. V.R. Khanolkar, Director, Indian Cancer Research Centre, Parel, Bombay-12.

Dr. N. Mookerji, Officer-in-charge, Leprosy Department, School of Tropical Medicine, Calcutta.

Dr. N. Figueredo, Superintendent, Acworth Leprosy Home, Wadala, Bombay.

Dr. P.L. Kapoor, Special Leprosy Officer, Bombay State, Office of the Surgeon General with the Government of Bombay. Bombay-1.

Dr. K.R. Chatterjee, Assistant Research Officer, Leprosy Department, School of Tropical Medicine, Calcutta.

Dr. R.V. Wardekar, Secretary, Gandhi Memorial Leprosy Foundation, Wardha. (Secretary)

Malaria and other Arthropod Borne Diseases Sub-Committee

Lieut.-Colonel Jaswant Singh, Deputy Director General of Health Services, New Delhi. (Chairman)

Dr. A.M. Francis, Assistant Director of Public Health, 81, Mount Road, Cathedral Post, Madras.

Dr. T. Ramchandra Rao, Assistant Director of Public Health (Malaria), Bombay State, Connaught House, Poona.

Dr. N.G.S. Raghavan, Assistant Director, Malaria Institute of India, Delhi.

Colonel N.D.P. Karani, Officer-in-charge, Hygiene Department, Armed Forces Medical College, Poona-1.

Dr. S.C. Seal, Professor of Epidemiology, All-India Institute of Hygiene & Public Health, Calcutta.

Dr. H. Trapido, Deputy Director, Virus Research Centre, Poona.

Dr. B. Ananthaswamy Rao, Offg. Director, Malaria Institute of India, Delhi. (Secretary)

Tuberculosis Sub-Committee

Dr. P.V. Benjamin, Adviser in Tuberculosis, Government of India, Directorate General of Health Services, New Delhi. (Chairman)

Colonel P.N. Bardhan, Professor of Pathology, Armed Forces Medical College, Poona.

- Dr. J. Frimodt-Moller, Medical Superintendent, Union Mission Tuberculosis Sanatorium, Arogyavaram, Near Madanapalle, South India.
- Dr. K.S. Sanjivi, Professor of Medicine & Physician, Madras Medical College and Government General Hospital, Madras.
- Dr. B.K. Sikand, Director, Tuberculosis Centre, Circular Road, New Delhi.
- Dr. C. Chandrasekharan, Professor of Statistics, All-India Institute of Hygiene & Public Health, Calcutta.
- Dr. K.N. Rao, Director of Medical Services, Andhra State, Hyderabad-Dn.
- Dr. P.K. Sen, Professor of Medicine, (Tuberculosis & Chest Diseases), Medical College, Calcutta.
- Dr. K.L. Wig, Principal, Medical College, Amritsar. (Secretary)

Venereal Diseases Sub-Committee

- Dr. R.V. Rajam, Director, Institute of Venereology, Government General Hospital, Madras-30. (Convener)
- Dr. C.W. Chacko, Serologist, Venereal Diseases Laboratory, Institute of Venereology, Madras Medical College, Madras.
- Dr. Sourin Ghosh, Professor of Surgery (Venereology), Medical College and Hospital, 88, College Street, Calcutta.
- Dr. K.C. Kandhari, Lecturer in Skin & Venereal Diseases, Medical College, Amritsar.
- Major-General S. Narain, Commandant, Armed Forces Medical College, Poona.
- Dr. K.V. Venkatraman, Serologist and Chemical Examiner to the Government of India. School of Tropical Medicine, Calcutta.

Sub-Committee for Standardization of Intelligence Tests

- Dr. B. Kuppuswamy, Professor of Psychology, Maharaja's College, Mysore. (Convener)
- Shri F.S. Chothia, Vocational Guidance Officer, Vocational Guidance Bureau, 3, Cruickshank Road, Bombay-1.
- Dr. D. Ganguly, Department of Psychology, University College of Science and Technology, 92, Upper Circular Road, Calcutta.
- Dr. S.K. Parukh, Consulting Psychologist, 646, Khareghat Road, Dadar, Bombay.
- Dr. K.C.K.E. Raja, Co-ordinating Officer, Central Demographic Teaching and Research Centre, Tata Institute of Social Sciences, Chembur, Bombay-30.
- Dr. T.V. Srinivasiah, Faculty of Education and Psychology, University of Baroda, Baroda.

Nutrition Education Sub-Committee

- Dr. K. Mitra, 3, Shashi Ghosh Lane, Calcutta-5. (Convener)
- Dr. V. Ramakrishnan, Assistant Director-General of Health Education, Directorate-General of Health Services, New Delhi.
- Dr. V.N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor.
- Dr. (Smt.) Muktha Sen, Professor of Maternity & Child Welfare, All-India Institute of Hygiene & Public Health, Calcutta.
- Dr. C. Gopalan, Deputy Director, Nutrition Research Laboratories, Coonoor.
- Dr. M.V. Radhakrishna Rao, Assistant Director-in-Charge, Department of Nutrition, Government of Bombay, Haffkine Institute, Parel, Bombay-12.
- Dr. (Smt.) H.M. Sharma, Assistant Director of Public Health, Madras.

Nutrition Survey Sub-Committee

- Dr. K. Mitra, 3, Shashi Ghosh Lane, Calcutta-5. (Convener)
- Dr. C. Gopalan, Deputy Director, Nutrition Research Laboratories, Coonoor.
- Dr. K. Someswara Rao, Nutrition Research Laboratories, Coonoor.
- Shri K.K. Mathen, All-India Institute of Hygiene & Public Health, Calcutta.
- Dr. V.N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor.

Sub-Committee on Protein Malnutrition

- Dr. V.N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor. (Convener)
- Dr. S.T. Achar, Professor of Pædiatrics, Madras Medical College and Government General Hospital, Madras.
- Dr. C. Gopalan, Deputy Director, Nutrition Research Laboratories, Coonoor.
- Dr. C. Chandrasekharan, Professor of Statistics, All-India Institute of Hygiene & Public Health, Calcutta.
- Dr. V. Subrahmanyam, Director, Central Food Technological Research Institute, Cheluvamba Mansion, Mysore.
- Dr. M.V. Radhakrishna Rao, Assistant Director, In-charge Department of Nutrition, Haffkine Institute, Parel, Bombay-12.

Sub-Committee for Compiling Data on Physiological Norms of Indians

- Dr. V.N. Patwardhan, Director, Nutrition Research Laboratories, Coonoor. (Convener)
- Dr. B.K. Anand, Professor of Physiology, All-India Institute of Medical Sciences, New Delhi.

Dr. J.D. Pathak, Professor of Physiology, Medical College, Baroda.

Dr. G.K. Karandikar, Professor of Pharmacology, Medical College,
Baroda.

Dr. J.C. Sachdev, Professor of Physiology, M.G.M. Medical College,
Indore.

**Sub-Committee for Compiling Information regarding Facilities
etc. on Physiological Problems in Medical Colleges.**

Dr. B.B. Dikshit, Director, All-India Institute of Medical Sciences,
Safdarjang, New Delhi. (Chairman)

Dr. J.D. Pathak, Professor of Physiology, Medical College, Baroda.

Dr. B.K. Anand, Professor of Physiology, All-India Institute of Medical
Sciences, New Delhi.

Dr. G.K. Karandikar, Professor of Pharmacology, Medical College,
Baroda. (Secretary)

III. TECHNICAL REPORT OF THE RESEARCHES CARRIED OUT DURING THE YEAR 1957.

The researches carried out during the year under report were recommended by the Scientific Advisory Board at its meetings held in Mysore on the 26th and 27th November, 1956, and were approved by the Governing Body of the Indian Council of Medical Research at its meeting held in New Delhi on the 23rd March, 1957. *The views expressed by the individual workers are not necessarily the views of the Council.*

CLINICAL RESEARCH

1. Clinical Research Unit under Dr. V. R. Khanolkar at the Indian Cancer Research Centre, Bombay.

1. *A Chemical method for estimation of chorionic gonadotrophic hormone (C.G.T.H.) in urine.*—The known biological methods for the estimation of C.G.T.H. are time-consuming and hence attempts are being made to develop a chemical method for their estimation. GTH being protein in nature, its quantitative estimation by means of the phenol reagent is being standardised. The details of the method are given in the report. The gonadotrophic activity of the hormone preparation obtained by this method is checked by a bioassay method. A mean concentration of GTH in male urine was found to be 5.11 ± 0.714 mg./24 hour (expressed as casein equivalent).

2. *Estimation of the non-protein nitrogen constituents of serum and urine in healthy subjects and in cancer with secondary liver and kidney dysfunction.*—A study on the non-protein nitrogen constituents of serum and urine in healthy subjects and in cancer cases with secondary liver and kidney dysfunction was undertaken. The purpose of the study was (a) to investigate whether the changes in serum and urinary levels of these constituents, as compared to those of healthy subjects, are characteristic of these diseases, and (b) to find out if any specific relationship existed in these diseases between the serum and urinary NPN constituents.

So far, 23 healthy individuals, 6 cancer cases with secondary liver dysfunction and 7 cancer cases with suspected kidney dysfunction have been studied for their serum and urinary NPN constituents. The six cancer cases with secondary liver dysfunction were characterised by the liver function tests. On the basis of the results of this study it appears that there is not an appreciable change in the serum NPN constituents in cancer cases with secondary liver dysfunction, but a high elevation in the same constituents is observed in cancer cases with suspected kidney dysfunction.

3. *Metabolic Studies on Thiosters of Hydrocarbons.*—Rats were fed with pure dibenzothiophene and their urine was collected. From the pooled urine, a colourless crystalline compound m.p. 257° (decomp.) and an oil have been obtained. Identification of these products is in progress.

2. Neurological Unit under Dr. V.R. Khanolkar at the Indian Cancer Research Centre, Bombay.

I. EXPERIMENTAL OBSERVATIONS ON LATHYRISM

In continuation of certain observations made last year further experiments were undertaken to test the hypothesis that the feeding of *Lathyrus sativus* could facilitate the noxious effects of neurotoxic agents in doses that would be innocuous to normal stock-diet fed animals. Four groups of young Wistar Rats including those maintained for a period of six months on either a 95 per cent or 100 per cent lathyrus diet and their respective controls, were challenged with graded doses of KCN administered as an aqueous solution by intraperitoneal injection, as follows :

Group (1) received 0.5 mg/kg. body weight, Group (2) 1.0 mg/kg., Group (3) 2.0 mg/kg. and (4) 3.0 mg/kg. body weight respectively per dose, by intraperitoneal injection. All the animals of Group (1) received a total of 8 injections (one per day), while the remaining three groups received 7 injections of the doses specified above. All the animals survived the full schedule of injections.

During periods ranging from one to three days after the last injection the animals were sacrificed, and the brain proximal portion of the cervical spinal cord, and portions of the liver were removed carefully for histological examination. Histological changes of varying degrees were found in the brains of all the animals. Pyknosis, shrinkage of cellular outlines, tortuosity of the dendritic processes and homogenous deep staining, with loss of differentiation between nucleus and cytoplasm or pallor with progressive degrees of disintegration of the cell body, were the main nerve cell alterations. There was no appreciable difference in the pattern of distribution of such changes in *Lathyrus*-fed or control animals.

A rough quantitative evaluation of the changes observed in each of these animals was attempted, making this evaluation as objective as possible. The extent of the changes in both nerve cells and white matter was used as a criterion to indicate these in numerically increasing grades of $N_{\pm}$, N_{+} etc. and $W_{\pm}$, W_{+} etc. The sign $+$ was given the value of 1, and $\pm$ of $\frac{1}{2}$ respectively to both nerve cell and white matter changes. The total obtained for each of the experimental and control groups was averaged and expressed in the terms of average numerical values.

It was found (a) that there was no distinct linear relationship between the extent of the changes observed and the doses of cyanide

administered, either in the experimental or control groups. (b) While the average numerical index of brain changes of the *Lathyrus*-fed groups as a whole and individually were slightly more than their respective controls, this difference was not considered significant.

While the results obtained so far, are not considered to, in any way, significantly offer support to the hypothesis on which this experiment was based, it is proposed to conclude these investigations with observations on similar groups of animals treated with increasingly higher doses of cyanide.

II. STUDIES IN PYRIDOXINE DEFICIENCY

(a) *Monkeys*: One of the two monkeys—Monkey No. P-1 maintained on the dietary pyridoxine deficiency for the last 21 months, has been sacrificed for histology. At the end of this period, the animal had lost five pounds in weight, looked anæmic and highly emaciated with an extensive loss of hair all over the body, particularly over the joints of the legs. There was a marked fall in the levels of blood hæmoglobin, vitamin B₆, plasma total protein, A/G ratio, and iodine number of fatty acids, and a rise in the plasma alkaline phosphatase value. However, the levels of blood urea, plasma total lipids, and total cholesterol remained unaffected. These results appeared to be indicative of damage of the liver.

The histological examination of the viscera of this animal is in progress.

Monkey No. P-2 did not show any marked changes clinically. However, significant biochemical changes have been observed in the levels of vitamin B₆, A/G ratio, plasma alkaline phosphatase, and iodine number of fatty acids. Although direct correlation between Vitamin B₆ and iodine number of fatty acids could not be seen during the course of the induced deficiency, the prolonged deficiency brought about a marked reduction in the plasma iodine number of fatty acids after a period of 19 months, in both the monkeys. In the absence of adequate supply of pyridoxine it appears, the animals are unable to make proper use of essential fatty acids.

An increase in blood urea levels observed in pyridoxine deficient rats (Beaton *et al*, J.B.C., 200 : 715, 1953) was not seen in monkeys.

(b) *Rats*: Experimental observations on induced pyridoxine deficiency in rats mentioned in our last year's report were continued.

Inbred, adult male Wistar Rats weighing between 180–250 gms. were used. The stock diet used is a modification of the diet employed by Tulpule and Patwardhan (I.J.M.R. 38 : 3, 1950) and Beaton *et al* (Arch. Biochem. Biophysics. 51: 94, 1954).

The vitamin B₆ contents of blood and liver were determined by the microbiological assay of Atkin *et al* (Ind. Eng. Chem. Anal. Ed. 15 :

141, 1943), liver and blood transaminase by that of Tonhazy *et al* (Arch. Biochem. 28 : 36, 1950), using Marsh *et al* technique for blood transaminase (J. Nutrition, 56 : 115, 1955). The estimation of urea was carried out by nesslerization using urease suspension, and the liver total lipids by the method of Channon *et al* (Biochem. J. 31 : 1736, 1937).

RESULTS :

(a) *Clinical*.—Early clinical manifestations of the deficiency such as roughness, patchy brown discolouration of the fur, reddening of the margins of the ear and skin of paws appeared in 1—2 weeks in both the pyridoxine deficient and deoxypyridoxine treated animals. There was however, a transient regression of these symptoms after 4—6 weeks. The severity of the affection of nose, paws and the anogenital region were observed at the end of $2\frac{1}{2}$ —3 months and 8— $8\frac{1}{2}$ months in the case of deoxypyridoxine treated, and pyridoxine deficient rats respectively. The delayed appearance of these symptoms may be attributed to the use of adult rats of 180—200 gms. This was necessary to have sufficient material for the entire biochemical studies. Using adult rats of same weight group, Ramalingaswamy and Sinclair, (J. Invest. Dermatology, 20 : 81, 1953) have observed that severe pyridoxine deficiency symptoms occurred after 6 months feeding of the rats on diet deficient in pyridoxine.

(b) *Biochemical*.—The effect of the induced deficiency by deoxypyridoxine appears to be similar to that of dietary pyridoxine deficiency so far as the levels of vitamin B₆ in blood and liver are concerned. While the levels of blood and liver transaminase diminish significantly in the case of deoxypyridoxine treated rats, only blood transaminase values fall markedly whereas the liver transaminase is not significantly affected in the case of pyridoxine deficient animals. In one set of recovery experiments where the animals were kept for 3 months on deoxypyridoxine diet and then fed pyridoxine for 4—5 weeks (Group IV), the levels of vitamin B₆ transaminase and urea reverted almost to normal. The deoxypyridoxine fed rats showed two-fold increase in blood urea while these levels were only slightly elevated in the pyridoxine deficient group, as compared to controls. Definite correlation could be seen between the levels of blood and liver vitamin B₆ and transaminase in the control and experimental groups. The values of vitamin B₆ compare favourably with those of Tulpule and Williams (J. Nutrition, 57 : 529, 1955) and those of transaminase are higher than those reported by Caldwell and McHenry, (*loc cit*).

There appears to be no accumulation of fat in the livers of these animals and in fact the values of lipids in experimental groups are lower than those observed in controls. These results are in agreement with those of Caldwell and others.

Histological examination of tissues of the rats sacrificed so far is in progress.

III. REFERENCE WORK

1. *Kyasanur Forest Disease*.—During the course of this year the unit received specimens of brains and viscera from post-mortems performed on three fatal human cases of Kyasanur forest disease in Mysore, and similar specimens from about 10 monkeys found dead in the forest. Histological examination of these specimens have been completed and the findings are being prepared for publication.

2. *Encephalitis material from Singapore*.—During the course of this year a large amount of material drawn from an epidemic of encephalitis from Singapore was received for study. These included assorted brain blocks from 36 cases, complete half brains with portions of viscera from another 12, and incomplete specimens from 6 others. The first lot has been examined and has revealed histological findings of a disseminated non-suppurative encephalitis, suggestive of virus encephalitis in the majority of cases. Two of these cases revealed intranuclear eosinophilic inclusions. The rest of the material is under study.

3. *Miscellaneous*.—A total of 135 specimens, inclusive of muscle biopsies, neurosurgical biopsies, and brains obtained from post-mortems on neurological cases, were received for study.

3. Clinical Research Unit under Dr. R.N. Chaudhuri at the School of Tropical Medicine, Calcutta.

I. CLINICAL AND FIELD WORK.

A. *Tropical eosinophilia* :

A clinical study of 100 indoor cases.—Most of the patients were males (90) between the ages 11 and 30 and residents of Calcutta. Occupation and environment did not seem to have any influence on the incidence. A past history of allergic manifestations due to rhinitis, asthma and dermatitis was present in 27 per cent of cases ; a few had allergic rhinitis while in hospital and nearly one-third of the patients gave history of some allergic disease among their family members. Exposure to cold was a common precipitating factor.

Clinical features.—The onset was usually insidious, but acute in a few: the duration of illness was from 3 months to 23 years. The common presenting symptoms were cough and/or bronchial spasm with or without expectoration. Other symptoms present in varying proportions were breathlessness, vague pains in chest, low irregular fever, loss of weight and occasional spitting of blood. Various non-pulmonary symptoms and signs, mostly gastro-intestinal, were present but apparently unconnected with tropical eosinophilia. Infected throat was present in 25 per cent of cases. Rhonchi and rales were common findings in the lungs with emphysema in a few, while the main radiological changes were mottling and/or increased broncho-vascular shadows with hilar reactions.

Laboratory examinations.—Wbc per c. mm., average 22,470, highest 43,000, lowest 6,200. Eosinophils per c. mm., average 13,934 (62 per cent), highest 35,360 (80 per cent), and lowest 2,088 (18 per cent). The common intestinal parasites were present in 21 per cent of patients. None had microfilaria in blood. Various other examinations were made, but the results were of no importance except that intradermal tests with *dirofilaria immitis* antigen were uniformly positive in all the 23 cases tested including 6 cases reported last year. In 5 cases the test was repeated after treatment with positive result. Eight control cases including 5 with microfilaria in blood gave negative results except one. With hydatid fluid it was positive in about half the cases, while with hookworm antigen obtained locally was uniformly negative. Biopsies of the liver, lymphnodes and skin showed nothing special except eosinophilic infiltrations. Spirochætes often found much in excess in bowel mucus and sputum, than in the control cases, were reduced after treatment. Blood of 104 family members of 31 patients were examined for eosinophilia of whom 11 had eosinophil above 20 per cent, maximum being 60 per cent in one.

In treatment, the preliminary report on diethylcarbamazine tried in 28 cases and submitted last year showed that with it the response was striking in 20 cases, the results being "indeterminate" in 5 and "failure" in 3. Of the failures 2 did not also respond to arsenic. The trial was carried out this year on a further series of 34 cases making a total of 62. The daily adult dose was 400-800 mgm. in divided doses for 5-7 days. The pulmonary symptoms usually disappeared within a week with a marked fall of eosinophil count in 2-5 weeks. Initial aggravation of cough and spasm occurred in 9 cases. Mild untoward reactions viz. nausea, pain in abdomen, slight fever, etc., were not uncommon, especially with larger doses, but did not necessitate discontinuance of the drug. Two cases however required a second course. The drug is quicker in action and efficient; next comes arsenical injections and after it carbarsone, aureomycin and antimonials. The last three are slow or unreliable. There was no advantage in combining diethylcarbamazine with carbarsone. Only 2 out of 5 cases treated with aureomycin, 0.5 gm., 6-hourly had complete relief. Prednisolone, as reported last year, had a temporary effect; it is very useful in cases with status asthmaticus. Antimony injections (I.V. or I.M.) were tried in 18 cases. They were not always tolerated well and the response was good in 6 of which one relapsed after a month. Spleen extract, sulphonamide, antihistaminics, urethane, penicillin and streptomycin were without effect.

The benign nature of the disease, long duration when untreated, relapses, absence of gross pathological changes and the fact that the condition is influenced by drugs with parasiticial (helminthic) action and not by antihistaminics are in favour of an infection with one or more types of organism of low virulence. History of allergy in 27 per cent cases and the usually prompt but temporary response to prednisolone suggest the possibility of an allergic factor.

A follow-up of 20 cases treated with diethylcarbamazine for a period of 3 to 12 months showed a further fall, on an average from 18 per cent

to 9 per cent. One relapsed after a year, but responded just as well as in the first attack.

(2) *Field Survey*.—In continuation of a preliminary survey on eosinophilia last year, in certain rural areas of West Bengal, further surveys on the incidence of eosinophilia among 'normal' people in certain areas of Assam and West Bengal were carried out. In Goalpara district blood of 530 members of all age groups were examined both for absolute eosinophil and differential count. Eighty cases had eosinophil count above 20 per cent up to a maximum of 74 per cent, the highest incidence being noted in young age groups. Thirty-two cases had absolute count between 2,000—15,200 (2,000—3,000 in 24; 4,000—5,000 in 6; 5,500 in 1 and 15,200 in the other), but none of them showed any clinical manifestation. In Jalpaiguri district 628 subjects were examined. Night blood was collected for microfilaria as well as for differential count in the same subject. Eosinophil above 20 per cent was detected in 115, 23 having a count of 30 per cent and above up to a maximum of 64 per cent without any clinical manifestations. In 39 cases microfilaria was found in blood of whom only six had eosinophil count between 20 per cent and 30 per cent. These results do not indicate obvious association of high eosinophilia with filarial infection, although failure to demonstrate parasite does not of course exclude the infection. In a Factory near Calcutta where workers are exposed to gaseous substances and dust, 70 showed eosinophils above 20 per cent (20—30 per cent in 59; 31—50 per cent in 8 and 51—65 per cent in 3) out of 981 adult males examined. Two gave past history of asthmatic attacks. It indicates that exposure to vapours, dust etc., does not cause any increase of eosinophils nor aggravates pulmonary symptoms in cases who had eosinophils above 20 per cent.

Comments.—Three surveys are reported on the incidence of eosinophilia (20 per cent and above up to 74 per cent) among 2,139 apparently 'normal' people (men, women and children) in certain urban and rural areas of Assam and West Bengal including a factory area. The overall incidence of eosinophilia was 12 per cent and most of the cases were found in younger age groups (up to 20 years of age). The survey indicates that massive eosinophilia can exist without any symptom. In one area (Jalpaiguri) 6.5 per cent of the population examined were found to have microfilaria in their blood, but only 6 out of 39 positive cases had eosinophilia between 20 per cent and 30 per cent; the others had less.

B. Tropical Splenomegaly:

(1) *A follow-up study*.—Analysis of all the data of 200 cases of chronic splenomegaly showed that they were of varied ætiology as reported last year. Some of them were due to post-hepatitis, portal cirrhosis, extra-hepatic portal obstruction, thalassæmia and metabolic disorders, but majority of them belonging to rural areas were sequel to chronic malaria, malnutrition being an associated factor, contributing to the hepatic changes which may lead to cirrhosis (aggravated by repeated antimony injections on mistaken diagnosis of kala-azar). A follow up study of 60 cases of tropical splenomegaly—43 treated medically and 14 after splenectomy, 3 having died soon after the operation was made, the total period of observation being 2 to 4 years.

In the non-operated series, 22 cases (4 years or less duration) in the 'early' stage were cured on suppressive anti-malarial therapy—weekly or fortnightly for half to one year. In the 'late' stage, 7 out of 14 patients (4—12 years' duration) there was remarkable reduction in the size of the spleen to 2" or less from 6"—9". Others remained in same state or became worse, one having died of hepatic failure following hæmatemesis. In the 'terminal' stage (8 to 18 years' duration), 3 out of 7 died and the remaining four had recurrent attacks of hæmatemesis. Seventeen cases in the late stage were subjected to splenectomy. Three died of operation, one of hæmatemesis and hepatic coma one year after and another from ascites and hepatic failure 2 years after while 5 were lost sight of. The remaining 7 patients complained of progressive weakness and lost weight by 4—15 pounds after the initial gain within one year although their blood picture was almost within the normal range. Two had hæmatemesis and melæna 1½ and 2½ years, after the operation. Irregular fever was complained of by 4 patients. Repeated needle biopsy of the liver showed necrosis of liver cells, cellular infiltration and fibrotic changes of varying grades in six of them, and the lesions were found to be more advanced than in the pre-operative biopsies. Careful assessment of a case especially with regard to the state of liver is therefore indicated before splenectomy.

It is of interest to note from the field study that as a result of anti-malarial measures for several years, the condition, hitherto a serious menace to public health in rural areas, has been virtually eliminated from the area.

(2) *Investigation for presence of abnormal hæmoglobin*.—Fœtal hæmoglobin was estimated in the Hæmatology Department in 32 cases, following the alkali denaturation technique of Singer *et al.* The average level of fœtal hæmoglobin was 2.12 per cent and the range varied from 0.0 to 4.4 per cent. Using an L.K.B. model paper electrophoresis equipment, electrophoretic analysis of hæmoglobin was done in 19 cases. All the patients showed a single spot having electrophoretic mobility of A hæmoglobin and the genotype in each case conformed to AA pattern. None showed any abnormal hæmoglobin.

C. *Spleen extracts*.—Acetone extract of bovine spleen was prepared in the laboratory and evaluated clinically in urticaria, bronchial asthma and tropical eosinophilia. Cases of urticaria showed variable improvement, a number of them being completely relieved during the period of observation for 3 months. Others had little or no improvement.

II. EXPERIMENTAL WORK

A. *Tropical splenomegaly*.—Continuation of the study showed that latent *P. berghei* infection in rat could cause chronic splenomegally of large size. Significant pathological changes in the liver, focal necrosis, cellular infiltration and fibroblastic proliferation, were also observed, and the fibroblastic activities were more marked if the diet was deficient. Initial deposition of malarial pigment in both the spleen and liver ultimately disappeared after 1½ to 2 years. The experimental findings

were thus somewhat similar to those in clinical cases. Control rats treated with mepacrine after each inoculation showed no significant splenic enlargement and hepatic involvement.

B. *Amœbiasis*.—Intra-cæcal inoculation of crude culture of *E. histolytica* in guinea-pigs produced acute intestinal amœbiasis, leading to death within a week or so. Some changes in the liver were also observed. The diarrhœic manifestation was, however, conspicuous by its absence in all these animals and, therefore, it was not always possible to examine the stool. The cæcal content at the time of sacrifice showed, however, presence of amœba in most of them. To facilitate repeated examination of the cæcal content for *E. histolytica*, without sacrificing the animal or surgical measure, a method was devised by application of a permanent hollow metallic fixture into the cæcum for easy inoculation as well as withdrawal of fæcal material for examination, whenever required.

4. Inquiry on the Endemic Focus of Schistosomiasis under Dr. H.I. Jhala at the Grant Medical College, Bombay.

Infection experiments on Snails.—About 15,800 snails (*Ferrissia tenuis*) were infected during the year with different doses of miracidia. A majority were infected en-masse. 10,500 *F. tenuis* collected from nature at Gimvi were infected at Gimvi and were brought to the laboratory. 1200 laboratory bred snails were taken to Gimvi in an atmosphere of oxygen, infected at Gimvi and were brought back to the laboratory. Samples of urine obtained from patients in Bombay showed considerable fluctuation in the number of viable ova, and in the degree of activity of hatched out miracidia.

The infected *Ferrissia tenuis* that survived a period of 5 weeks after infection emitted few cercariae. A yield of limited number cercariae was available time and again. This however, was too less for animal infection. Due to this the infected animals were found to be negative after a period of three months.

It was observed that a majority of the infected snails die in the laboratory because of the infection and partly due to the failure of air-compressor plant. *F. tenuis* are very small in size and quite delicate. It is also observed that these snails are not good host capable of prolific liberation cercariae. At Gimvi it is found that the rate of infection in *ferrissia* is around 2 per cent in general.

Indo-planorbis exustus and a good number of *Limnæa luteola* were also infected in the laboratory with *S. haematobium* but majority of the snails did not survive the period of incubation. Those that survived were found negative on dissection.

Animal infection :—

(a) With *S. haematobium* :

Different methods of animal infection were tried on mice, young rabbits, but these gave negative results as the cercarial yield was very poor.

(b) With *S. Spindalis* :

Mice and rabbits were infected with cercariae emitted by naturally infected *Indo-planorbis exustus* and *Limnæa Luteola* snails. A good number of *S. Spindalis* adult worms were recovered after three months of infection. The cercarial yield from these snails was considerable.

Survey of the village Gimvi.—Survey of School children at Gimvi was carried out in the month of January, 1957. Out of 29 cases examined 18 were with viable ova (out of these six had gross haematuria), Seven with non-viable ova, and 4 were negative.

It was observed that the population of *ferrissia tenuis* was excellent where the structure of the river bed is stoney, water level is shallow, and when there is a constant movement of human beings and animals.

Experimental work.—*Indo-planorbis exustus* were used in experiments on quantitative cercarial yield, and the effect of heat, light and salinity in different grades on the emission of cercarial.

Experiments were also carried out on phototrophicity of cercariae, —viability of *F. tenuis* in different conditions of oxygen requirement in an atmosphere of oxygen.

Experiments on the viability of *S. hæmatobium* ova under different conditions of media and temperature totally failed. Staining, clearing and mounting of adult worms (*S. spindalis*) was done satisfactorily.

Re-Survey of village Gimvi. —House to house survey of Gimvi was carried out in first week of September 1957. In this survey people born, and brought up in the village, and no casual visitors were also taken into account. The total population worked out to be 933 out of which 468 were males and 465 females, 676 per male were staying in the village and 257 were casuals at the time of survey.

Infection was maximum in Lower-Marathawada. About 40 per-cent of males staying in Lower-Marathawada had been infected some time or the other. The incidence was maximum in the age group of 5—15 years, and the 5—25 years group was found to be the usual period of incidence.

It was observed that a majority of cases treated by the Public Health Department, Poona, in May, 1955 showed relapse. Three fresh

cases of infection were found in Lower-Marathawada and one in Upper-Marathawada.

A paper on the detailed survey work is in the press.

GENERAL

Foreign workers were consulted about certain aspects of the research work but no satisfactory reply was received from any of them. An invitation to attend the African Conference on Bilarsiasis held at Brazzaville on 26th November to 8th December, 1956 was received. An express letter was written to Director, Indian Council of Medical Research if the Council or W.H.O. would finance the deputation of one of the members of this enquiry. This was not arranged perhaps due to short notice.

A research scheme presented by Dr. A.K. Varma of the Live Stock Research Station, Bihar was well commented upon by us.

5. Inquiry into the use of artificial hypothermia (hibernation) in open intracardiac surgery under Dr. P.K. Sen at the Seth G.S. Medical College, Bombay.

Altogether ten experiments have been carried out since 1st August 1957. This work has been divided in two main heads :—

A. *Studies in extreme hypothermia.*—To carry out open 'dry' cardiectomy on completely non-pulsatile 'standstill' heart with a view to exclude cerebral anoxia, ventricular fibrillation and air embolism. A group of seven experiments, to study the behaviour of the heart at low temperatures ranging from 22°C. to 10°C., have been carried out.

B. A further group of three experiments was conducted to produce a partial cooling to about 32°C., and producing chemical cardiac arrest by perfusing the coronary circulation with potassium-citrate solution through the ascending aorta after outlet clamping.

A. EXPERIMENTAL PROCEDURES

Adult mongrel dogs were anaesthetized with intravenous pentothal (30 mg. /kg. body-weight), an endotracheal tube was inserted and the lungs hyperventilated (48 times per minute). Venesection was done and intravenous drip started. Electro-cardiographic control tracings were taken, and animals were immersed in ice-cooled water (at temperature 0 to 4°C.) after recording the original temperature with rectal electric thermo couple. The rectal temperature and E.C.G. records were maintained every ten minutes. When the required lower temperatures were obtained the cold water was siphoned off, and replaced by tap-water. The temperature of bath was raised to 45°C. by adding warm water to re-warm the animals to their original temperatures.

Out of the seven dogs, three went into complete cardiac arrest (Nos. 4, 7 and 15), and did not register any beat after re-warming.

Dog No. 5 when cooled to 10°C., registered 4 beats per minute. On re-warming, it developed ventricular fibrillation at 27°C., and died. Dogs Nos. 9, 12 and 17 registered less than 10 beats per minute when re-warming was started. There was full recovery of the heart beats, reflexes returned and automatic breathing started, but all died within three hours of revival.

B. EXPERIMENTAL PROCEDURE

Adult mongrel dogs were anaesthetized with intravenous pentothal (30 mg./kg. body weight), an endotracheal tube was inserted and ventilated at a rate of 16 per minute. Electrocardiographic control tracings were taken. The original temperature was recorded with rectal electric thermo couple and animal immersed in ice-cooled water. The temperature and E.C.G. were recorded every ten minutes till the temperature fell to 32°C. The animal was taken out of the bath and placed on the operation table. After drying, the animal, the chest was opened by a transverse incision at the level of 4th intercostal space. The sternum was cut across and retracted. The pericardium incised. The azygos vein was isolated and ligated. The superior vena cava and inferior vena cava were isolated and clamped, and lastly the ascending aorta was clamped. A solution of potassium citrate in blood was injected in the aorta, proximal to the clamp to perfuse the coronary arteries till the heart stopped in diastole. After four minutes of complete cardiac arrest, plain blood was injected into the aorta, proximal to the clamp, under pressure, to wash out the potassium citrate from the coronaries. The clamp was released. The heart started beating, the beats being weak initially and showing some fibrillary twitchings. Calcium chloride was injected in the aorta after applying the clamps again when the heart beats improved but were not well sustained.

In these three experiments different concentrations of potassium citrate (25 per cent, 10 per cent, 5 per cent) have been used, these being of the nature of early pilot procedures. The optimal concentration of the cardioplegic drug varies with many factors including that of temperature.

6. Effect of metaphyseal stimulation of longitudinal bone growth under Dr. B. Mukopadhyaya at the P.W. Medical College, Patna.

This series of experiments have been carried over from the year 1954 through 1955 to 1956 and 1957. On the basis of observations made in 1954 and 1955, it was felt that the failure to obtain increase in length of a growing bone could be explained on the basis of a comparatively transient increase in the vascularity of the region of the metaphysis produced by the implantation of thin soft autogenous grafts taken from

the same young animal. In order to make the process of increase in vascularity more sustained, hard cortical adult bone removed from normal healthy adult goats and preserved by chemical sterilization was used in the present series as the grafted material. It was thought that such grafts will take longer to be absorbed and will thus produce more prolonged increase in vascularity. The process of absorption of the graft was followed by serial X-rays and in order to avoid cessation of the vascular response repeated grafting was performed in the present series of experiments. The following observations were made :--

- (1) Even repeated grafting and sustained increase in the vascularity of the diaphyseal side of the epiphyseal cartilage does not produce acceleration of the rate of proliferation of the cartilage columns and does not produce any permanent increase in the length of the growing bone.
- (2) Repeated grafting very definitely produces sustained increase in the vascularity of all the layers of tissues, i.e. deep fascia, the muscle, the periostum of the region of the metaphysis of the long bones.
- (3) Repeated grafting also produces an inflammatory response and this is accompanied and followed by replacement of fibrosis causing adhesion of different layers of tissues adjacent to the joint and thus may be a factor in ultimately producing rigidity of the joint.
- (4) Repeated grafting appears to have caused varying degrees of direct injury to the epiphyseal growth plate leading to either retardation or cessation of growth in the cartilage plate.
- (5) Repeated grafting is technically a difficult procedure and unless performed with extreme caution may lead to pathological fracture and thus cause a deformity of the limb.

It appears wrong to equate increase in the vascularity of the region of the metaphysis to a stimulating agent of the epiphyseal growth plate. The other point to note is that the metaphyseal side of the epiphyseal growth plate is not really the proliferating section of the growth plate and thus increase of vascularity of this area may not stimulate rapid multiplication of cells.

7. Electro-Retinography in Vascular Hypertension under Dr. R.P. Dhanda at the M.G.M. Medical College, Indore.

The earliest stage of the work on this enquiry is to standardise the normal electro-retinogram of Indians over the age of 40 years and study variations if any with the younger age group already reported from this E. R. G. Unit in the years 1952-53.

During period under report 26 normal eyes of patients over the age of 40 years were investigated only those cases were selected whose vision

was not poorer than 6/18, there was no lens opacity, fundi were normal except the usual senile changes expected in a person of this age group and the blood pressure was within normal limits (i.e. 140/90). It may be stated that it is not very easy to collect normal subjects fulfilling these conditions in the out-patient Department particularly over the age of 40 years. In each case the vision was recorded, ophthalmoscopic examination was done and blood pressure was noted.

The patient was dark adapted for 5 minutes and electro-retinograms were recorded on a Sanborn Cardiograph machine by the method already established at this E. R. G. unit at Indore. The stimulus of 1/4 seconds of an intensity of 80 lux and at a interval of 15 seconds was maintained in all cases in this investigation of normal Indians.

The average b-pot of 26 eyes of 22 normal Indians above the age of 40 years has been calculated to be .29mV which is distinctly less by .03mV when compared to .32mV of the normal persons of the age group 15—40 years. This subnormal electro-retinogram in older age group may be due to the senile changes at this age.

The table on this page gives the details of the observations in each case :

Serial No.	Name	Age	B. P.	b-pot in mV	
1	S.K.	40	106/70	R.E.	.4/7
2	B.R.	48	130/80	R.E.	.137/10
3	S.K.	40	138/90	R.E.	.4/10
4	B.R.	45	112/76	R.E.	.3/15
5	G.R.	46	126/88	R.E.	.25/12
6	M.K.	55	120/76	R.E.	.3/15 L.E. .4/12
7	G.S.	50	132/76	R.E.	.3/12
8	B.R.	47	100/70	R.E.	.32/14
9	R.B.	50	112/74	R.E.	.345/11
10	V.H.	53	124/80	R.E.	.30/8
11	M.G.	55	112/78	R.E.	.30/11
12	V.D.	50	130/86	R.E.	.250/6 L.E. .222/9
13	B.L.	50	118/84	R.E.	.2/7 L.E. .2/7
14	G.B.	50	110/70	R.E.	.111/9
15	S.L.	42	124/90	R.E.	.266/3
16	R.	52	110/66	R.E.	.2/6
17	J.B.	40	116/76	L.E.	.315/8
18	R.B.	50	120/84	R.E.	.411/13
19	V.P.	57	110/70	R.E.	.308/6 L.E. .306/8
20	L.M.	65	140/70	L.E.	.255/16
21	R.C.	48	128/82	R.E.	.30
22	S.D.	55	118/82	R.E.	.302/9

This E. R. G. Unit has already established during the last 5 years that the electrical potential recorded from the normal retina of Indians between the ages of 15 and 40 years is .32mV.

Stimulus to this work on the clinical application of electro-retinography in vascular hypertension was provided by the experimental work by Henkes who reported that the electro-retinogram was found to vary with sudden lowering and raising of blood pressure in animals. It is now an established fact that arteriolar changes in retina and kidney, are an essential part of Pathology of essential hypertension and therefore it was considered interesting to study by electro-retinography the eyes of patients suffering from benign and malignant hypertension. This can easily be understood because the associated changes in blood vessels will affect the nutrition of retina which in turn will effect the function capacity of retina and ultimately altering the electrical potential produced at the level of the retina.

With this object in view of the work on ENQUIRY ON VASCULAR HYPERTENSION has been planned to be completed in the following stages :—

1. Recording electro-retinogram in normal Indians above the age of 40 years and to compare the results with those already reported in the earlier years of the work at this E. R. G. unit.
2. Study the effects on the electro-retinogram of increasing the sensitivity of retina by varying the dark adaptation again in the normal Indians over the age of 40.
3. Study the changes if any in the electro-retinogram in cases of different grades of vascular hypertension and correlate these findings with the visual and ophthalmoscopic findings and the clinical records of the medical wards.
4. Study the effects of treatment on electro-retinogram and thus, if possible, to assess the prognostic value of electro-retinography in vascular hypertension.

8. Inquiry entitled "Biochemical and Serological investigations on *Escherichia coli* and *Klebsiella* strains isolated from urinary infections" under Dr. S.P. Gupta and Dr. N.P. Gupta at the Medical College, Lucknow.

(1) *Collection of strains*.—1036 strains of coliform organisms isolated from established cases of urinary infection have been so far collected in this department. They have been preserved in one per cent agar stabs.

(2) *Biochemical reactions*.—So far 900 strains have been tested for their biochemical reactions with 27 media. The result indicate that on the basis of the pattern of biochemical tests, about 60 per cent were *Escherichia coli*.

The use of KCN broth to differentiate *Escherichia* from *Klebsiella* was continued. Strains belonging to *Escherichia coli* did not grow in KCN broth in 24 hours while *Klebsiella* grew well in the medium. This test was however not found specific, as about 7 per cent of *Klebsiella* grew after 48 hours and about 21 per cent of *Escherichia* strains were found to grow in the medium in 24 hours.

(3) Hæmagglutination tests were continued with these organisms. 345 strains of *Escherichia* were tested with red cells of sheep, rabbit, rat, man, chicken and guinea pig. Of the 345 strains of *Escherichia coli* tested, 164 strains were found to cause agglutination of red cells from one or several animal species. The guinea pig cells were most frequently agglutinated, while sheep and rabbit cells were agglutinated by least number of strains. Red cells from rat, man and chicken occupied an intermediate position. Many strains agglutinated only one species of red cells. Human red cells alone were agglutinated by 34 strains, guinea pig cells alone by 21 strains, rat cells alone by 15 strains, and chicken cells alone by 12 strains. Six strains agglutinated rabbit cells only and 2 strains agglutinated only sheep cells. 5 strains were found to agglutinate all the six species of red cells tested. The remaining 69 strains gave variable reactions.

There was no correlation between the hæmagglutinating property and the serological type of *Escherichia* strains.

The hæmagglutinating strains were tested in the living state for the presence of L-antigen with unabsorbed 9 'OL' sera. 179 hæmagglutination positive strains have been tested so far. 92 strains out of 179 did not show any agglutination with these sera. 87 strains could be typed and their L-antigen could be determined. Maximum number of strains were grouped under L-antigen 1.

(4) Test for hæmolysin were carried out with all the *Escherichia* strains and it was found that 58 strains possessed this property. There was no significant correlation between the hæmagglutinating property and capacity to produce hæmolysis except in 5 strains out of 449 strains of *Escherichia* which produced hæmolysis and agglutinated guinea pig red cells. Hæmolytic activity was not confined to any well defined serological group.

(5) *Serological studies on strains of Escherichia*.—(a) Determination of O-group of *Escherichia* strains with the help of 25-O-sera (constituting the diagnostic schema of Kauffmann, Knipschild and Valene) after preparing the antigen at 100°C. for one hour and again at 120°C. for 2½ hours have been continued.

(d) Determination of L-antigen of *Escherichia* strains.

Sera against 15 L-antigen were prepared in rabbits and preserved in neutral glycerol. Determination of L-antigen has so far been carried out with unabsorbed 9 'OL' sera, 316 strains of *Escherichia coli* were tested for the presence of L-antigen in living state by slide agglutination

method. 127 strains out of 316 could not be typed with these nine sera. It was also noted that these strains did not belong to 25-0-groups constituting the diagnostic schema. 34 strains were auto-agglutinable. Maximum number of strains possessed L-antigen 1 and L-antigen 18.

Thus 44.93 per cent strains of *Escherichia coli* could be indentified serologically with the available 9 'OL' sera for the presence of L-antigen. Tests with further sera are in progress.

9. Investigation into the role of allergens and various other factors in the production of bronchial asthma in Rajasthan in general and in Jaipur area in particular under Dr. R.M. Kasliwal at the S.M.S. Medical College, Jaipur.

The pollination calendar of a place is a record of the time in days and months when pollens capable of causing allergic manifestations are present in the atmosphere. It will be realized that the first step towards distinguishing local allergenic plants is to obtain a thorough knowledge of the local vegetation as well as of the time when they effloresce. It is also equally clear that the problem is as much botanical as medical. To accomplish this work a complete botanical survey of the area in and around Jaipur city has been done and the period when the vegetation effloresces has been recorded. The newer additions to the flora, investigated already, is given below :—

Trees.—*Acacia arabica*, *Albizzia odoratissima*, *Phoenix sylvestris*, *Cassia marginata*, *Cassia siamea*, *Cassia surettensis*, *Cassia javanica*, *Eucalyptus tereticornis*, *Bauhinia tomentosa*, *Melia azedarach*, *Salix tetersperma*, *Holoptelia integrifolia*, *Phyllanthus emblica*, *Polyalthia longifolia*.

Weeds.—*Cyperus rotundus*, *Amarantus viridis*, *Bulbostylis barbatus*, *Cyperus compressus*, *Cyperus iria*.

Grasses.—*Dactyloctenium scindium*, *Saccharum spontaneum*, *Cenchrus catharticus*, *Elusine verticillata*, *Zea mays*, *Cenchrus biflorus*, *Panicum paspaloides*, *Imperata cylindrica*.

The anemophilous pollens and the abundant entomophilous ones have been collected during the period of their pollination. A pollen library has thus been established. The pollen library has materially helped towards identifying the pollens on the catchment slides.

A daily census of the ærial pollen has been carried by the Gravity slide technique using the standard sampling device (Durham). An attempt was made to identify the individual pollens on the catchment slides. The following pollens were identified in the catchment slides :—

Trees.—*Acacia*, *Callistemon*, *Leucæna glauca*, *Malvaceæ*, *Morus alba*, *Dodonæa viscosa*, *Daucus carota*, *Mimoseæ*, *Holoptelia*, *Azadirachta*, *Albizzia*, *Delonix*, *Phyllanthus*, *Putranjiva*, *Leguminosæ*, *Compositæ*, *Cassia*, *Phœnix*.

Weeds.—*Amarantus-Chenopod*, *Argemone*, *Abutilon*, *Datura*, *Ricinus*.

A correlation was done with the field survey and this double checking has resulted in pin-pointing the actual time in days and months when the pollination would be clinically significant from the point of diagnosing and treating an allergic individual.

The influence of the meteorologic conditions on the concentration of pollens as a whole has been studied. It was seen that the pollen count is effected by a number of factors, such as rainfall, humidity, temperature, duration of sunshine and the velocity of wind. A shower of rain tends to decrease the pollen counts, while a period of draught and the rise of temperature following a heavy rain is associated with increased pollen counts. Increased humidity seems to diminish pollen liberation and is associated with a decrease in pollen counts and vice versa. The rainy season in this part starts with the arrival of monsoon in the 4th week of June. The results of field survey showed that the vegetation effloresces from about mid of July but the catchment slides have shown that the maximum concentration in the atmosphere is attained some two months later because the rainy season persists during this time and the increased humidity interferes with the liberation of pollens.

To increase the allergenic flora prevalent here, mass clinical testing has been performed at an Allergy Research Clinic set up in the S.M.S. Hospital, Jaipur. All the patients of respiratory allergy are referred to at this clinic. With the help of a thorough history taking, clinical and laboratory investigations, an attempt is made to discover the offending allergen or allergens in these patients and then the I.C. skin tests are performed. So far 80 cases of respiratory allergy, including cases of asthma, allergic rhinitis, hay fever and allergic bronchitis have been investigated. Pollens of *Azadirachta indica* (Neem) ; *Amarantus spinosus* (Kateli chaulai) ; (*Dodonaea viscosa*; *Holoptelia integrifolia* (chilli) ; *Phyllanthus emblica* (Amla) ; *Argemone mexicana* (Kateli) ; *Chenopodium album* (Bathuwa) ; *Ricinus communis* (Arand) ; *Chloris barbata* ; *Cynodon dactylon* (Dub) ; *Desmotachya bipinnata* (Dab) ; *Digitaria marginata* (Jada) ; *Elusine indica* ; *Pennisetum orientale* ; *Sorghum halepense* (Baru) have been considered to be definite offenders. A more definite information would, however, be available after testing a larger number of patients.

Of the 80 cases of allergy, 49 cases were found to be of pollen allergy, and hyposensitization treatment has been started in these cases. The success or failure of the treatment would take sometime to evaluate and more definite conclusion could be deferred till such time.

10. Inquiry on the formation, distribution, structure and development of the splanchnic nerves under Dr. Inderjit at the Medical College, Amritsar.

1. The origin, formation, distribution and termination of all the three splanchnic nerves have been followed on both sides in 94 instances

(47 cadavers). Fine branches have been followed with the aid of a dualoupe.

2. Similar dissection has been made in one full term foetus.

3. The histological structure of the nerve has been examined by making serial sections in the case of one full term foetus. Sections were stained with appropriate stains.

4. Complete serial sections of the two embryos have been prepared. Staining methods used include Masson's trichrome, H & E and de Castro. These will enable us to study the development of splanchnic nerves.

5. Detailed findings have been recorded on a specially prepared proforma. Photographs of important and unusual findings have also been taken.

11. Inquiry on the value of commercial silk grafts to bridge large blood vessel gaps under Dr. Yudhveer Sachdeva at the Medical College, Amritsar.

Transplantation of preserved aortic homografts in the abdominal aorta below the origin of renal arteries has been performed in 30 dogs. Grafts were taken from abdominal and thoracic aorta of dogs and were preserved in the following media :—

	Dogs
(i) 4 per cent neutral formalin solution at room temperature	10
(ii) 70 per cent ethyl alcohol at room temperature	10
(iii) Balanced salt solution containing 10 per cent homologous serum and antimicrobial drugs at a temperature 2°C. to 6°C.	10

Neutral formalin solution was prepared by adding an excess of calcium carbonate to a 4 per cent solution of formalin and then filtering it. Grafts were preserved in this solution at room temperature. Seventy per cent ethyl alcohol was prepared by diluting absolute alcohol and grafts were preserved at room temperature. Grafts to be preserved in balanced salt solution were taken aseptically and the solution was cultured for the presence of pathogenic organisms before transplantation.

FORMALIN PRESERVED GRAFTS

In the first group, aortic homografts preserved in 4 per cent neutral formalin solution were used. In all 10 such grafts were studied in dogs weighing from 6.4 kg. to 11.9 kg. aortic segments were preserved from 6 to 28 days. The animals were sacrificed at varying intervals and changes in the graft studied.

Results.—In four animals post mortem was done 5, 11, 55 and 59 days after the operation (grafts were preserved for 19, 14, 11 and 11 days, respectively). There was no complication and the grafts were functioning well. The graft in the dog sacrificed after 55 days showed a few small thrombii.

320 days after transplantation of the graft, one of the dogs with 28 days preserved grafts is still under observation. It has good femoral pulsation.

Two dogs died of late hæmorrhage due to dehiscence of the anastomosis. One died after 4 days and the other after 7 days. Grafts transplanted were preserved for 14 and 9 days, respectively.

Two dogs developed paraplegia 2 days and 3 days after operation. Grafts were preserved for six days and 17 days, respectively. On post mortem the aorta was found to be occluded by a thrombus in each case.

In one dog femoral pulsation was absent but there was no paraplegia. Post mortem was done after 52 days of transplantation and the graft was completely occluded by two thrombii at the two ends of the graft (Graft was preserved for 14 days).

II. *Alcohol preserved grafts.*—Ten grafts were used in dogs weighing from 6·4 kg. to 13·6 kg. Time of preservation of the grafts varied from 2 to 48 days.

Results.—In three dogs post mortem was done 10, 32 and 44 days after the operation. Grafts were preserved for 1, 13 and 16 days, respectively. Grafts were found to be patent. Two dogs with grafts preserved for 3 days and 7 days are still under observation (178 and 147 days after transplantation of the graft). In one dog post mortem was performed after 82 days of operation (39 days preserved graft was used). There was a thrombus occluding the aortic lumen. There was no paraplegia. One dog died of hæmorrhage 5 days after operation due to dehiscence of the anastomosis (48 days preserved graft being used). Three dogs developed paraplegia and post mortem was done after 2, 14 and 19 days after transplantation. (Grafts used were preserved for 2, 15 and 10 days, respectively). On post mortem all the three grafts were found to be completely thrombosed.

III. *Grafts preserved in balanced salt solution.*—Ten such grafts were transplanted in dogs weighing from 6·4 kg. to 13·6 kg. Grafts were preserved from 1 to 35 days.

Results.—Five dogs are still under observation after 63, 31, 20, 15 and 12 days after transplantation of the grafts. Grafts were preserved for 3, 6, 4 and 5 days respectively. In one dog post mortem was done 47 days after operation (5 days old graft was used). The animal had no paraplegia, but a thrombus was found occluding the aortic lumen. In another dog post mortem was done 4 days after the operation (2 days preserved graft was used). The graft was patent. Two dogs died of hæmorrhage due to dehiscence of the anastomosis

2 days and 3 days after transplantation of the graft. Grafts were preserved for 13 days and 27 days, respectively. Two dogs developed paraplegia, 3 and 11 days, respectively after operation. Grafts preserved for 10 and 2 days, respectively, were used. On post mortem aortic lumen was found to be occluded by a thrombus.

Commercial silk grafts.—Tubes made of Kashmir silk have been used in 4 dogs. The tubes were made in such a way that the inner surface was smooth and the ends were everted to prevent fraying of the threads. The tubes were autoclaved. For some time before transplantation the tubes were soaked in the blood of the animal being operated and allowed to dry.

Results.—One dog died of hæmorrhage due to dehiscence of the anastomosis after 5 days of transplantation. Three dogs are still under observation after 73, 8 and 2 days of the transplantation of the prosthesis.

12. Study of renal changes following ureteric ligation and estimation of recovery following release of obstruction by ureteric transplantation under Dr. B.N. Balkrishna Rao at the G.R. Medical College, Gwalior.

Technique of study.—1. Incomplete obstruction is being produced by introduction of Laminaria tent 2.0 cm. long, through the left ureteral orifice in the bladder after opening the bladder. After various duration of time it is contemplated to perform nephrectomy on the right side accompanied by release of obstruction. Before release of tent, a retrograde pyelographic record to be kept.

2. Sudden, complete obstruction in the ureter is produced by surgical ligation of left ureter at the level of the lower pole of left kidney.

3. Work on production of so-called idiopathic hydronephrosis is still in the preliminary stages.

4. The lymphatics of normal and hydronephrotic kidney and ureter is being visualized by injection of 1 c.c. of 11 per cent of patent blue into the wall of the ureter. A graphic record of lymphatics seen as distended blue streaks is being kept. This is being done to elucidate (if any) the role of lymphatics in absorption of hydronephrotic sac contents have been tubulovenous and pyelovenous backflow. Both have been observed during this inquiry.

5. The electrophoretic pattern of serum at various stages is being done. The densitometric and photographic record of strips obtained, are being maintained.

6. The kidney function is studied by injecting either 10 c.c. of 0.4 per cent indigocarmine or 6 mg. (1 c.c.) of phenolsulphonphthalein (P.S.P.)

7. Blood urea estimation is being done using King's technique.

8. Photographic records are being maintained at each stage of both kidneys.

DISCUSSION

It has been recorded from the work of the last two years, that restitution to nearly normal of hydronephrotic kidney of certain duration is possible. The recognition of this fact answers to some extent the queries usually posed :—

(i) For how long an obstructive lesion of the ureter, e.g. a calculus, which is causing complete or incomplete obstruction, be treated in a conservative manner ?

(ii) In what cases recovery of a hydronephrotic kidney is possible.

Our results so far differ fundamentally from previously published reports of restitution to normal of hydronephrotic kidney.

The knowledge gained through experimental work so far, has following important therapeutic applications :—

(i) Conservative treatment of ureteral obstruction may be followed without any increased risk for much longer than is believed at present.

(ii) The indications for nephrectomy need not be so frequent in cases of obstructive hydronephrosis. It should only be performed if sufficient time has elapsed after removal of the obstruction to allow the renal parenchyma optimum facilities for recovery.

The production of hydronephrosis by incomplete obstruction has produced many practical difficulties. The use of a Laminaria tent in the ureter, at least has the merit of producing a gradual obstruction, though incomplete. This state closely, if not accurately, simulates obstruction by a calculus which is growing in the ureter.

13. Inquiry into pneumoconiosis in the non-industrial population of Rajasthan with special reference to emphysema under Dr. R.K. Goyal at the S.M.S. Medical College, Jaipur.

The occurrence of early pneumoconiosis in the apparently normal individuals of Rajasthan has already been reported to the I.C.M.R. These lesions in the non-industrial population can be found only in the desert regions. The lungs of apparently normal rabbits, guinea-pigs

and white rats in Rajasthan have not shown the existence of pneumoconiosis. Interstitial pneumonitis can be due to infective causes alone, hence the presence of this lesion exclusively is not important from the point of view of diagnosis. Granulomatous lesions associated with the presence of silica particles are much more diagnostic. A histopathological examination of the lungs of other uninjected animals which could be used for laboratory work gave the following information :—

Three monkeys caught fresh from the jungle showed the presence of granulomatous lesions varying in extent.

One of these monkeys was emaciated, a number of greyish-white plaques were found in the lungs on naked-eye examination.

On microscopic examination, definite granulomatous lesions containing giant cells and dust cells were present.

Focal emphysema as well as thickening of some of the branches of the pulmonary artery were also found.

An examination of the lungs of twelve stock dogs showed varying degrees of granulomatous lesions in eight out of them.

Four dogs did not show definite evidence of pneumoconiosis, the lesions, were slight or absent in the dogs aged 7 to 8 months.

Out of the four goats examined, the one aged 7 months did not show any granulomatous lesions, the others aged 1 to 4 years had a few dust cells or dust particles in the alveolar walls, and varying degrees of interstitial pneumonitis were present in all these cases.

Seven stock sheep aged 1 to 2 years were also examined.

In addition to the presence of different degrees of interstitial pneumonitis, four had dust cells or dust particles in the alveolar walls.

In a preliminary experiment, whilst the lungs of a pig aged 7 to 8 months showed the presence of granulomatous lesions plus a few dust cells in the alveolar walls, the lungs of another pig aged 2 to 2½ months old showed slight granulomatous lesions, but no dust particles in the alveolar walls. It can, therefore, be concluded that monkeys, dogs and perhaps pigs, are very susceptible to pneumoconiosis. Sheep and goats are less susceptible, whereas white rats, rabbits and guinea-pigs are rather resistant.

Inhalation experiments.—A preliminary set of experiments had to be put up to determine the best method of producing pneumoconiosis in the experimental animals, and the results were as follows :—

Rabbits.—Five rabbits were made to inhale dust every day for 4 to 6 hours in the inhalation-chamber for 9 to 22 days.

One rabbit which inhaled dust for 9 days continuously and was sacrificed 12 days after the commencement of the experiment did not develop granulomatous lesions.

The other rabbits which were made to inhale the dust discontinuously with long intervals of rest and were sacrificed from 5 weeks to 5 months from the date of commencement of the experiment, developed lesions of early pneumoconiosis; three of them showed thickening and narrowing of arteriolar walls.

Guinea-pigs.—When one guinea-pig was made to inhale dust on alternate days for 10 days in all, and was sacrificed one month after the commencement of the experiment, no granulomatous lesions were discernible. When the inhalation was repeated in two other guinea-pigs after an interval of three weeks for a second period of 10 days, and the animals were sacrificed 3 to 5 months after the commencement of the experiment, granulomatous lesions varying in degree with a perivascular and sub endothelial proliferation of connective tissue in a number of pulmonary arterioles were seen.

Rats.—The dust inhalation was either given daily or on alternate days for total periods varying from 6 to 64 days. The animals were sacrificed from 8 days after the commencement of the inhalation up to period of 7 months. Out of the 18 rats exposed, nine had slight lesions, five had moderate lesions and four had no granulomatous lesions. The results were rather irregular, the extent and type of the lesions could not always be correlated with the total period of the dust inhalation.

Monkeys.—Eight monkeys were exposed to inhalation of dust for periods varying from 10 to 38 days in all, they were sacrificed 13 days to 5 months after the commencement of the experiment. All of them showed granulomatous lesions varying in extent and containing dust cells, a definite thickening of the walls of some branches of the pulmonary artery was seen in four cases.

Two baby monkeys born in the Pathology Department were exposed in the inhalation-chamber for 7 and 11 days, respectively.

The first one was sacrificed after $2\frac{1}{4}$ months and the other after $3\frac{1}{2}$ months.

The first monkey had very slight lesions, and the second had definite granulomatous lesions with focal emphysema.

These experiments were carried out to produce fairly marked lesions, as the preliminary experiments had shown that apparently normal monkeys in Rajasthan suffer from early pneumoconiosis. It appears that there is another factor responsible for the presence of marked lesions, this factor might possibly be an infective one.

The control experiments in which charcoal and calcium sulphate, etc., are to be used for inhalation are in progress.

Size of quartz particles.—A microscopic examination of human lungs carried out with the help of chemical polaroids revealed the presence of doubly refractile quartz particles at places where an aggregation of carbon particles was present. The size of the quartz particles did not

exceed 5μ . In the case of lungs of monkeys, most of the quartz particles did not exceed 5μ , but particles of 10μ in length were occasionally encountered.

The ordinary atmospheric air in Rajasthan does not cause pneumoconiosis in rabbits, guinea-pigs and white rats. When these animals are exposed to artificial dust-storms intensively in the inhalation-chamber, then alone they develop early lesions.

It is, therefore, apparent that dust storms constitute an important factor in the causation of pneumoconiosis. The microscopic examination of dust removed from the tops of almirahs showed the presence of a high percentage of particles which were less than 5μ in diameter, but all of them did not have the refractive index of quartz. The minute particles had also mostly lost their crystalline structure.

They constituted roughly 10 to 30 per cent of the dust in different samples.

14. Investigations of certain Ayurvedic preparations in treatment of Diabetes Mellitus under Dr. R. V. Sathe, at the Grant Medical College and Sir J. J. Group of Hospitals, Bombay.

The investigations in the use of Ayurvedic drug : "Jasad Bhasma" (Zinc) were being done in the J. J. Hospital since April 1955, under the Indian Council of Medical Research Scheme. The procedure of investigation adopted has been detailed in the report for the year 1955-56. This year 21 cases are reported out of which 8 are from the last year's report and so have been followed for nearly two years. The rest of the cases from the last year's report could not be traced. The remaining 13 are cases treated during the year of report and are under observation for nearly one year. Much of the clinical material investigated has been wasted, as the diabetic patients come irregularly for a follow up. Out of 21 cases which are thoroughly followed-up during the year under report, three had tuberculosis, two had fistula in ano, two were suffering from diabetic neuropathy and two had diabetic retinopathy as complications. Fourteen patients were using insulin of one type or other and the dose varied from 16 to 104 units a day. They were also on restricted diet—not taking sugar, rice and potatoes—but it was not properly calculated. The age group of the patients, when they came for treatment was : five were under forty years, while sixteen were above forty one years. The age of onset of the disease on the other hand showed that 8 persons had their onset below the age of 40 ; while 13 had it after 41 years.

THE DURATION OF DIABETES

Under	1 year (recent onset)	...	3
Between	1 to 5 years	...	14
Between	6 to 10 years	...	2
Above 10 years		...	2
			21

The diabetic cases can be divided, clinically into three groups :

(1) Mild—3 (2) Moderately severe—5 (3) Severe—13 out of these, 9 had complications as mentioned above.

THE RESULTS OF THE TREATMENT

These results with "Jasad Bhasma" can be summarized as under :

(1) *Urine Volume*.—The 24 hour urine excretion which was above normal, came to within normal limits, as reported in the last year's report.

(2) *Urine Sugar*.—The 24 hour urine sugar before treatment with Jasad Bhasma, varied from trace to 218 gms. a day. After treatment with Jasad Bhasma, it was reduced to nil in 13 cases, varied between 6 to 8 gms. in 5 cases, between 11 to 15 gms. in 2 cases and to 18 gms. a day in one case.

(3) *Blood Sugar Level*.—The blood sugar levels of all cases under treatment with Jasad Bhasma were lower than before as can be seen from the following table. For convenience cases under observation over one year are grouped as A, while those under observation for one year or less are grouped as B. Only fasting blood sugar levels are given below for convenience.

Fasting Blood Sugar	Before Treatment		After Treatment			
Level.	Group A.	Group B.	Group A.	Group B.		
80 to 120 mg%	Nil	Nil	3	+	2	= 5
120 to 160 mg%	1	2	2	+	6	= 8
161 to 200 mg%	2	3	2	+	3	= 5
201 and above.	5	8	1	+	2	= 3
	—	—				—
Total	8	+	13			21
	—	—				—

It will be observed that in eight cases which have been followed up from the last year's report (Group A) there was relapse of diabetic condition in two cases, due to no proper control, and in one case due to tuberculosis. The remaining cases continued to be better than before. In Group B, except two cases all showed definite improvement in the carbohydrate tolerance, after treatment with Jasad Bhasma. One case with diabetes and tuberculosis showed improvement very well, being aglycosuric with fasting blood sugar at 125 mg. per cent, without insulin. The original fasting blood sugar in this patient before Jasad Bhasma treatment was started was 315 mg. per cent and the 24 hour urine sugar excretion was 60 gms. (Case No. XV).

BLOOD UREA BLOOD CHOLESTEROL

The blood urea and blood cholesterol readings were found to be within normal range throughout the period of observation under Jasad

Bhasma treatment in all cases. No adverse effects on urea and cholesterol levels in blood were observed when the diabetic patients were under Jasad Bhasma treatment.

HAEMATOLOGIC STUDIES

There is appreciably little change in hæmatologic studies except the reduction of hemoglobin values in some cases. Dietary inadequacy in the class of patients who attend the hospital may be one of the causes of subclinical findings in hemoglobin levels.

WEIGHT

In group A during the period of observation for over one year, five persons added weight from 4 to 11 lbs : and three lost by 4 to 10 lbs. In Group B : during the period of observation seven added weight by 2 to 14 lbs and five lost by 3 to 7 lbs, and one remained stationery. It will be seen therefore that out of the 21 cases reported 12 i.e. 57.0 % added weight and 8 i.e. 38 per cent lost weight.

INSULIN

Before treatment with Jasad Bhasma, six patients in Group-A were taking insulin and the dose varied from 16 to 104 units a day. After treatment with Jasad Bhasma only two cases required the help of insulin as they were of young age group.

In Group B, seven patients, out of thirteen were taking insulin, the dose varied from 24 to 80 units. After treatment with "Jasad Bhasma", out of these seven cases, six did not require the help of insulin, while one did not react to Jasad Bhasma treatment. Out of the 21 cases reported three did not react to Jasad Bhasma treatment and so required the help of insulin. Sugar excretion in 24 hours, with "Jasad Bhasma" and without insulin in 18 cases was as under :—

Sugar excretion in 24 hours - Nil in 12.			
„	„	„	Trace to 10 gms in 4.
„	„	„	11 to 20 gms in 2.

Six cases treated with Jasad Bhasma only without insulin at any time showed definite reduction in blood sugar level and urine sugar excretion.

DIET AND JASAD BHASMA

This point was discussed in last year's report. Diet alone had not shown appreciable change in blood sugar level in some diabetic cases. This year two cases are discussed to illustrate the point. It was observed in a control case that even though the patient was kept on a diabetic diet (Case No. 17) for 27 days his fasting blood sugar level was lowered from 231 mg. per cent to 202 mg. per cent only. So diet for 27 days did not show appreciable change in lowering of blood sugar level.

Another patient of severe diabetes (Case No. 20) with fasting blood sugar level of 300 mg. per cent was kept on diabetic diet for 21 days without any other treatment. Her fasting blood sugar level lowered down to 280 mg. per cent—a difference of 20 mg. per cent only. The patient was kept on the same diet and “Jasad Bhasma” was started. After six weeks of “Jasad Bhasma” treatment the fasting blood sugar level lowered down from 280 mg. per cent to 180 mg. per cent a difference of 100 mg. per cent. The patient added 4 lbs., in her weight also. This showed that “Jasad Bhasma” had a definite beneficial effect on carbohydrate tolerance.

Whenever Jasad Bhasma was stopped and the patient remained on the same diet, increase in blood sugar level was observed after some variable period. This showed definitely that Jasad Bhasma in some way helped carbohydrate utilization.

THE PLACE OF “JASAD BHASMA” IN THE TREATMENT OF DIABETES MELLITUS.

It has been observed that “Jasad Bhasma” acts better in maturity onset type of diabetic patients. Patients with onset of diabetes in young age group are less amenable to oral therapy, and more liable to relapse.

The effect of Jasad Bhasma on the diabetic syndrome was observed usually after 4 to 6 weeks. It varied with the individual case; depending upon the severity of the disease, accompanying complications and also the age group of the patient. Earlier the diabetes was detected and properly treated better the results. Uncomplicated cases reacted much earlier and in most satisfactory manner. Infection and irregularity in diet upset the carbohydrate metabolism, but Jasad Bhasma treatment improved the same.

Case No. XV - M.P.—A patient of severe diabetes with tuberculosis is worth mentioning. This case required 80 units of insulin a day. The initial blood sugar level here was 315 mgm. per cent. With Jasad Bhasma for four months she became aglycosuric and the fasting blood sugar came down to 125 mg. per cent.

It has been observed that some cases did not react to Jasad Bhasma treatment, and no definite cause could be ascribed for this. Diabetes is not a very simple problem and no definite cause for insulin inadequacy can be ascertained in each and every case. But it is worth while studying the effect of these minerals in Ayurvedic form, Jasad Bhasma, etc., as their cost is much less and no side effects have been observed thus far in cases treated for nearly two years. Another aspect of this treatment is that even though the drug takes a long time to act—it does not act immediately and rapidly like sulfonyl-urea compounds—the effects are long lasting. This can be seen from the case reports given in the appendix.

15. Inquiry on the metabolic fate of gelatin administered as a plasma substitute under Dr. C. Sivaraman, Senior Scientific Officer, National Chemical Laboratory, Poona.

Preliminary experiments were carried out to study the effects of transfusion gelatin of known average molecular weight on red cell, Hb and plasma protein concentrations, W.B.C. count, hæmatocrit, erythrocyte count, sedimentation rate and coagulation time in adult albino rats maintained on the laboratory stock diet. The efficiency of gelatin in the treatment of shock was also determined on the basis of the survival rate of rats. Experiments were carried out with dextran using albino rats as experimental animals. The results obtained were identical with those obtained with gelatin in that the hæmodilution caused by dextran infusions resulted in a decrease in erythrocyte count, hæmatocrit, and Hb and plasma protein concentrations. With dextran administration, however, the sedimentation rate and coagulation time were slightly higher than those obtained with gelatin transfusion. Although systemic allergic reactions were not noticed, the efficiency of dextran in combating shock as determined by the survival rate of rats bled to nearly half their blood volume and transfused with an equal volume of dextran was found to be comparatively lower than with gelatin. In a few instances, dextran was thought to be the immediate cause of death due to anaphylactic shock although evidence for this is not conclusive.

The physico-chemical studies were carried out for purposes of characterization of the transfusion gelatin prepared on a pilot plant scale.

The next step in the investigation was directed towards testing this material for pyrogenicity, toxicity and allergic and antigenic reactions.

All animals tolerated parenteral administration of gelatin and manifested no alterations in their physiological well-being. All animals gained weight and showed no anorexia.

Nutritional Evaluation.—The next phase of the investigation was designed to determine whether intravenous gelatin could be anabolically utilized. The studies were conducted on two groups of albino rats maintained on a low protein vegetarian diet containing 5% rice protein. After depleting the protein stores of the animals by maintenance on the diet for two weeks, one group of animals received, in addition to the diet 2 c.c. of gelatin intravenously twice a week for three weeks, the control group receiving by vein normal saline in place of gelatin. Experiments were now in progress to determine whether transfusion gelatin supplemented with amino acids administered orally as well as intravenously would promote utilization of the transfusion material for protein synthesis.

Results of Retention Tests.—These experiments were carried out on doubly-depleted dogs and rats and on normal rabbits. Gelatin concentration in blood and in urine was determined according to the method of Jacobson and Smyth (*Arch. Int. Med.*, **74**, p. 254, 1954). Hæmatocrit

was determined after centrifuging the heparinized blood samples in Wintrobe's tubes for 30 minutes at 3,000 r.p.m. Blood volume was estimated by injection of T-1824 Evans blue dye according to the method of Metcalf, Cutting and Favour (*Amer. Jour. Physiol.*, **141**, p.695, 1944). Nembutal was used as the anaesthetic at a level of 30 mg/kg. body-weight.

Histopathological Study.—Opinion is divided as to whether plasma expanders, such as dextran, polyvinylpyrrolidone (PVP), gelatin etc. cause anatomic changes in the internal organs. This aspect of the problem is of considerable importance in connection with the use of gelatin for transfusion purposes. However, anatomic changes were conspicuous in one specimen only. The structural alterations appear to be similar to the transient changes in the kidney reported by Popper *et al* (1945) following gelatin administration. To elucidate this point, further experiments are in progress. Histochemical tests with biopsy specimens (Morehead and Little, 1945) were all negative indicating that there was no deposition of gelatin in the tissues.

16. Development of a simple method of preserving or preparing aortic and arterial homografts under Dr. Timothy Takaro and Dr. A.C. Fletcher at the Wanless Tuberculosis Sanatorium, Wanlesswadi.

The experimental method consisted of the implantation of grafts 3 cm. to 5 cm. in length in the abdominal aortas of mongrel dogs. The graft material consisted of (a) homografts fixed and preserved in formalin, (b) grafts made of flexible, corrugated braided nylon tubing, (c) homografts fixed and preserved in 70 per cent ethyl alcohol.

Fifteen dogs each have been used for formalin-fixed and nylon grafts, and five dogs for alcohol-fixed grafts. Formalin-fixed grafts are technically satisfactory to handle and early results have been good. However, when animals were sacrificed 5 to 12 months after operation most of the grafts exhibited calcification and degenerative changes. The nylon grafts were technically more difficult to handle, but the late results have been superior. The grafts have remained patent and the lumen is found lined with a layer of intima.

Alcohol-preserved homografts have been reported upon by Kimoto, *et al.* (1954) and by More and Kajikuri (1956). This technique has been adopted recently and only five dogs have been operated upon. There were two early deaths, due to rupture of the graft, possibly a result of improper preservation. The remaining three animals have not yet been sacrificed.

The use of homografts, preserved in formalin or in alcohol, has the advantage over nylon grafts, that complicated segments, such as the arch of the aorta, are easily prepared and kept ready for use when needed. It seems likely that alcohol may prove preferable to formalin as a preserving agent.

17. Inquiry on bio-microscopical study of the conjunctival vessels in relation to the general arteriosclerosis and coronary artery disease under Dr. K.N. Mathur, Dr. K.S. Mathur and Dr. P.N. Wahi at the S.N. Medical College, Agra.

A total number of 215 cases were examined of which 80 were normal cases, 95 were Hypertensive cases and 40 coronary disease cases.

Biomicroscopical study of the conjunctival vessels was done by the technique perfected last year. Eighty normal, 95 hypertensive and 40 coronary diseases cases were examined.

Ninety-five of hypertension were studied. It was found that in hypertensive cases with the increase of blood pressure, the elasticity of the conjunctival blood vessels became progressively lesser, and abnormal tortuosity, loops, sharp bends, aneurysms and irregularities in the vessel calibre tend to increase. The lessening of the elasticity of the conjunctival vessels was manifested by the lesser extent of the maximum dilatation and in taking more time to reach the maximum dilatation, and in coming back to normal. The duration of maximum dilatation was also greater.

It was noted that in some hypertensive cases the maximum dilatation occurred comparable to that attained in the normal cases of the same age group, but time taken to return to normal was more, while in other hypertensive cases though the extent of dilatation was less the time taken to return to normal had not altered.

Forty coronary disease cases were examined. All of these were confirmed by electro-cardiogram. The elasticity of the vessels had no direct relation with the coronary disease. It was only indirectly related, depending upon the blood pressure of the case.

In 27 cases of hypertension blood cholesterol was determined, and it was found elevated in 21 cases only. Blood pressure of these cases ranged between 150 mm. and 230 mm. of Hg.-systolic and 85 mm. and 160 mm. diastolic. All the cases showed decrease in the elasticity of conjunctival vessels, according to the range of hypertension. Blood cholesterol had no direct relationship with the elasticity.

In all cases of hypertension fundus examination was done under homatropine. Fundus findings were positive in 54 per cent of the cases. Lowering of the conjunctival vessels elasticity was found in all cases whether they had any fundus finding or not.

18. Inquiry on histo-pathological study of trachoma in relation to the clinical stages of the diseases under Dr. Pashpati Nath Wahi, and Dr. P.N. Awasthi, S.N. Medical College, Agra.

During the last and the present year 83 biopsies have been registered out of 165 cases of trachoma. The control cases so far studied are so few in number that it will not be possible to comment upon.

Clinical Material.—The clinical material is provided by the In-patient and Out-patient Departments of the S.N. Hospital, Agra. The medical students also volunteered to act as control cases. Six groups of cases are being studied, based on the 4 clinical stages of Maccallan.

Biopsies.—The biopsies are taken both from upper and lower fornices after infiltrating the lids with 2 per cent novo-caine. A stitch is passed just above the lid margin and the lid so is everted. A chalazion forcep is applied on the everted lid so as to include the tarsal plate. A vertical incision is given on the tarsal plate and an incised piece of tarsal plate including conjunctival epithelium is removed and fixed in 10 per cent formal saline. The biopsy piece is processed and cut and stained with haematoxyline and eosine.

Observations and results.—172 cases have been registered up to 30th September, 1957. They comprised 10 asymptomatic cases and 158 cases of trachoma, 3 cases of chalazion and one case of spring catarrh. The age of the patients ranged from 10 to 70 years. The clinical diagnosis did not give much difficulty in well established cases. Asymptomatic and early cases presented difficulties which were finally labelled by clinicopathological data. Most of the cases came from poor and middle class, only one percent of cases were from higher class.

Histopathological diagnosis in 168 cases in various clinical stages has been done.

Smear examination findings.—As described by Linder, we came across two types of inclusion bodies; (1) initial bodies, and (2) elementary bodies. Initial bodies were seen in 66 per cent of total cases so far registered, whereas elementary bodies were noted in few cases.

Therapeutic response of various drugs in trachoma.—62 cases of trachoma in various stage were treated by the drugs. Two drugs were tried, viz. achromycin in oil locally, and sulphamezathine orally. Achromycin in oil was used as local drops every two hours during the day only. Sulphamezathine was given orally. The dose was 4 tablets start, and 2 tablets 6 hourly, the total dose was 25 g.

Thirty cases have been treated by achromycin in oil and the rest treated by sulphaemzathine.

19. Investigations into the factors affecting the susceptibility of diabetes to infection under Dr. T.P. Bharadwaj at the Topiwala National Medical College, Bombay.

The following studies were undertaken :-

1. Blood corticoid levels of diabetics have been estimated. These reveal significant difference from normal.

2. Control normal rabbits and rabbits rendered diabetic by alloxan, were given intraperitoneal injections of T.A.B. suspension. The rate of rise of antibody titre was noted by agglutination technique for over 2

months. Another group of rabbits was divided into two batches — a control normal and diabetic group. To both of them (the animals selected were of approximately equal weights) large quantities of antibodies against T.A.B. were given. The initial titres were noted and the rate of their dissimilation was followed by twice weekly agglutination reaction. This has been followed, up to the present for about 2 months. In neither instance have significant differences been noted. The studies are being pursued further.

3. Titres of T.A.B. agglutinating antibodies have been studied in 50 normal individuals without any history of clinical typhoid in recent past (three years). Similar studies have been started in diabetes.

4. Studies on opsonin, complement levels and certain metabolic aspects of leukocytes are in progress in normal and diabetic animals and men.

20. Inquiry on biochemical studies on tumor under Dr. S.C. Roy in the Department of Applied Chemistry, Calcutta University, Calcutta.

During the period under review the work has been continued with human malignant and other tissues and directed mainly towards the following lines :—

(1) Isotopic studies, (2) study of the tissue with respect to (a) free and protein amino acids, (b) glycolytic phosphate esters, (c) α -keto acids, (d) nucleic acids and PNA-P/DNA-P ratio, (e) dehydrogenase systems in the mitochondria, and (f) peptides of the tissue after the partial hydrolysis.

(1) *Isotopic studies.*—*In vitro* utilization of C^{14} -l-acetate by malignant and analogous normal or fibroid tissue has been followed up in different fractions obtained chemically and separated chromatographically, wherever necessary into different constituents: (a) respiratory CO_2 , (b) volatile compounds, (c) carbohydrates, including Kreb's cycle intermediates, (d) lipids and (e) free and protein amino acids. Preliminary scanning has been done to detect the compound having incorporation of C^{14} , and for more accurate determination recourse has been taken to radioautography.

Comparatively heavier incorporation of C^{14} has taken place in the following amino acids in malignant tissue: Glutamic acid, serine, glycine, aspartic acid, alanine, arginine and histidine, lysine, threonine, leucine, proline, methionine sulfoxide, tyrosine. From the values of the radiation count, it appears very likely that the incorporation is rather specific and not a general one. Radioautographic study which is under way will give more specific indications. Lipid fraction from the malignant tissue has also been very heavily incorporated as compared to that of the normal.

(2) (a) *Free and protein amino acids*.—Sixteen more specimens of tissue-malignant, fibroid or normal have been analyzed with respect to free and total amino acid contents. The results have been found, more or less, the same as reported last year.

(b) *Glycolytic phosphate esters*.—Ten different specimens of tissue (malignant, fibroid and analogous normal) have been analyzed and the results agreed to a considerable extent with those reported last year.

(c) *α -keto acids*.—Twelve specimens of tissue have been analyzed with respect to pyruvate, oxalacetate and α -ketoglutarate by the chromatographic method. The acids have been identified and the process for quantitative determination has been standardized and is being applied.

(d) *Nucleic acid and PNA-P/DNA-P ratio*.—Ten specimens of tissue have been analyzed and PNA-P/DNA-P ratio determined. Attempt has been made to isolate the DNA from human tumor tissue and separate the purine and pyrimidine bases chromatographically.

(e) *Dehydrogenase systems in the mitochondria*.—Mitochondrial preparation has been obtained from the tissue and glutamic acid, alanine and glycine dehydrogenases have been detected by using TTC (2,3,5-triphenyl tetrazolium chloride) as the electron acceptor.

(f) *Peptides from the partially hydrolyzed tissue*.—Proteins isolated from the tissue were subjected to partial hydrolysis, high speed centrifugation and chromatography of the different fractions so obtained. Several peptides have been located and will be studied in more details with the help of column chromatography. (Moore and Stein, *Jour. Biol. Chem.*, 211, p. 893, 1954).

21. Inquiry on synthetic media of tissue culture and the measurement of proliferation of the cells under Dr. C.V. Ramakrishnan, Faculty of Medicine, Baroda University, Baroda.

The conditions have been standardized to grow chick embryo tissues directly on glass in roller tubes using different media, such as HES, M₁₅₀, Eagle's medium, etc.

Several trials of experiments carried out so far show that the heart fibroblast explant can be grown in roller tubes in a purely synthetic medium.

Micro-methods have been standardized to estimate the sugar uptake, protein, total number of cells and DNA of the explant during the proliferation of the explant and a correlation has been obtained between these and the proliferation. Once the proliferation stops, the sugar uptake also stops simultaneously.

Preliminary experiments carried out show that if glutamine is omitted from the medium, the tissue proliferation is better. The evidence has been obtained to show that glutamine, when added, gets converted to glutamic acid and excess glutamic acid may come in the way of proliferation.

22. Clinical study of neuropathies under Dr. P.N. Chuttani at the Medical College, Patiala.

A total of 178 cases of various types of neuropathies have been studied, at first at Amritsar and later at Patiala; 171 have been analyzed in detail, the remaining 7 not having stayed long enough for a thorough study. It was found that the main ætiological or clinical groups met with were diabetic neuropathies in 34 patients, arsenical neuropathies in 19, Landry-Guillain-Barre syndrome in 18, subacute combined degeneration in 17, meralgia paræsthetica in 19, shoulder girdle syndrome in 7, nutritional neuropathy in 10, and the ubiquitous 'idiopathic' variety in 16. A large number of other varieties were also seen, such as those due to diphtheria (4), leprosy (3), typhoid (3), serum or post-vaccinal (3), alcohol (2), compression (2), pneumonia (1), brucellosis (1), bronchogenic carcinoma (1), cancer penis (1), acute idiopathic porphyria (1), hyperemesis gravidarum (1), carbon-monoxide (1), and amyloidosis (2). Cranial polyneuritis (2), neuropathy associated with Pink disease (1), and polyarteritis nodosa (1), were also met with and a single example of chronic progressive neuropathy was seen.

Apart from routine laboratory investigations, nerve biopsies from digital nerves of the involved limbs were available for histopathological examination conducted after staining of paraffin sections with H & E, azocarmine, osmic acid or myelin stain and of frozen section with silver stain. Frozen sections were also studied under polarized light. Changes, such as increased cellularity, thickening of perineurium and/or endoneurium increase of fibrous tissue, fragmentation, granularity, ballooning and absence of myeline and swelling, fragmentation, diminution or absence of axons were seen in various combinations. No specific histopathological picture emerged for any ætiological variety and there was no obvious correlation between the severity of histological change and the clinical severity of the neuropathy. However, pathological changes were the rule when neuropathy was well developed. A few serial biopsies from the same patients were also studied.

23. Inquiry entitled 'Relative value of rest and movement in the treatment of intra-articular fractures : an experimental study' under Dr. B. Mukopadhyya at the P.W. Medical College, Patna.

1. *Object of the Work.*—To study by animal experiments the relative values of the treatment by rest and treatment by early movement in cases of operatively induced intra-articular fractures and thus determine from such experimental studies the correct method of treatment of such fractures in human beings which occur as a result of accident. The treatment of such fractures in human beings is still a controversial issue and the evidence on which the different methods of treatment are based is entirely clinical.

2. *Conclusions of the work done so far.*—So far operations have been performed in three different joints of goats, the joints being the wrist, the knee and the ankle. Only these joints were selected as it is not possible to immobilize the other joints in goat. The plan of work

has been slightly altered since the submission of the report last year. The cases have been grouped into three types of fractures for each joint, as follows :-

- (1) Linear fracture without displacement.
- (2) Linear fracture with displacement of various degrees.
- (3) Comminuted fractures with multiple fragments and marked displacements.

The experiments have been so designed that for each joint immobilized after operation the same joint in a normal animal has been immobilized for the same period of time and in exactly the same fashion. For the present study the period of immobilization has been kept fixed at 4 weeks.

It will be seen from the study so far made that in intra-articular fractures of moderate severity without comminution, immobilization of the joint very definitely prolongs the period of disability. Encouragement of early physiological activity in the traumatized joint not only reduces the period of disability considerably but also produces more perfect end results, the movement in the joint returning to full normal range. With immobilization of the joint, a certain percentage of cases develop varying degrees of stiffness in the joint permanently. This difference in the results occur with only 4 weeks immobilization—a period too short to produce complete union in the fracture.

Where an intra-articular fracture is severely comminuted causing gross degrees of mal-alignment of the articular surfaces, perfect restoration of function is not possible, whatever method of treatment is used.

24. Inquiry on tropical pulmonary eosinophilia under Dr. M.V. Chari, at the Kasturba Medical College, Mangalore.

1. A report is given on the age, symptoms and laboratory findings of 33 males and 4 females treated under this inquiry from the 1st December, 1956 to 30th September, 1957 at the Government Wenlock Hospital, Mangalore.

2. Particular reference is made to the low grade temperature with raised eosinophil count.

3. It was observed that the paroxysmal attacks bore no relation to peripheral eosinophils.

4. Bone-marrow study was undertaken in 13 cases and was followed up in 6 cases. The results of this study are not conclusive.

5. The radiological findings of 41 cases with changes noted in the appearance of the skiagrams of 20 cases followed up are given.

6. Treatment was given with (a) Hetrazan (b) Banocide, (c) Entacyl, (d) Carbarsone, (e) Hetrazan and carbarsone, (f) Antimony, (g) T.A.B. vaccine, and (h) Strontium bromide. With the exception of the last a few patients responded to treatment both clinically and hæmatologically with each of the above lines of treatment.

25. Inquiry to investigate the mechanism of antibacterial action of specific antiserum under Dr. J.C. Ray at the Indian Institute for Biochemistry and Experimental Medicine, Calcutta.

Since the last report work on one of the aspects, namely, the effect of anti-serum on the lactose utilization by nucleotides stimulating adaptation in anti-serum treated cells is nearing completion.

(a) *Effect of antiserum on lactose utilization.*—It has been observed that the anti-serum treated non-adapted cells, after being pre-adapted to lactose, would utilize lactose to a much lesser degree as compared to those exposed to normal serum or the lactose adapted cells.

2-4-Dinitrophenol in a concentration of $10^{-3}M$ was found to inhibit the utilization in the adapted strain to 80 per cent as compared to the control ones. But the effect with methylene blue reduction was different. DNP was not at all inhibitory, on the contrary it unfolded some hitherto unnoticed non-specific reduction of methylene blue through to a very small extent. This prompted the investigators to search for another redox dye which will be more specific in reduction. At the present moment, 2-3-5-triphenyl tetrazolium chloride is being used as a substitute and preliminary experiments have shown it to be a highly efficient tool for bacterial adaptation studies as required in this investigation.

(b) *The effect of adenosine triphosphate (ATP) and other nucleotides in stimulating adaptation in anti-serum treated cells:* The progress of this part of the work was retarded considerably due to non-availability of the nucleotides in pure forms. It has recently been taken up and is being studied using the tetrazolium technique.

26. Studies on metabolism of the catechol amines in bronchial asthma and tropical eosinophilia under Dr. H.S. Chakravarti at the Indian Institute for Biochemistry & Experimental Medicine, Calcutta.

Scanty reports are available on the pattern of catechol amine excretion in various disease conditions, specially where physiological discharge of adrenal medullary hormones or their defective utilization in the body may be suspected, such as in bronchial asthma and in tropical eosinophilia. Aetiology of the symptom complex of tropical eosinophilia, still remains obscure. High count of blood eosinophils and occasional improvement with ACTH and corticosteroids, rather suggest a possibility of some dysfunction of the adrenal glands. It is well known that both the cortical and medullary hormones have eosinopænic effect, the later working through the pituitary-adrenal axis.

Considering all these facts, it was decided to investigate both the cortical and medullary function of the adrenals in cases of tropical eosinophilia and bronchial asthma. Investigations were made to find out the pattern of catechol amine excretion and 17-ketosteroid excretion in 24-hour urine, before and after treatment of tropical eosinophilia and bronchial asthma. Attempts were also made to reproduce tropical

eosinophilia in guinea-pig which, however, failed. Plasma protein pattern by paper electrophoresis were also studied in these cases.

CATECHOL AMINES EXCRETION IN 24-HOURS URINE.

In tropical eosinophilia.—Patients with characteristic symptoms of tropical eosinophilia were investigated clinically, radiologically and hæmatologically in the department for confirmation of the diagnosis. In a typical case, massive leucocytosis with eosinophils not less than 2,000/cu. mm. of blood were considered for selection. All the patients included in this work came to us before any specific treatment with arsenicals. They were subsequently treated as ambulatory patients with organic arsenical preparation (acetylarsan).

For normal control, 26 healthy individuals from laboratory workers were selected. They were ordinarily considered as free from any disease and did not show any high initial eosinophil count.

Urine collection.—Twenty-four hour urine collections were made with 5 c.c. of 12 N HCl as preservative. When immediate extraction was not possible, samples of urine were stored at 4°C. in a refrigerator for not more than 7 days. In cases of tropical eosinophilia, estimations were done before instituting any treatment and again one week after completion of the treatment with arsenical.

Extraction.—According to a slightly modified method of Euler 200 c.c. aliquot of urine with 20 c.c. of concentrated HCl was boiled for 20 minutes in a reflux condenser and filtered. To the filtrate was added 3.75 g. of aluminium oxide and alkalinized with concentrated NaOH solution till a pH of 8.5 was attained. A magnetic stirrer was used to shake the mixture continuously during alkalinization. The mixture was taken in a Buchner funnel on two sheets of filter paper and put to mild suction and the filtrate was discarded. The adsorbed amines on the alumina in the funnel was washed with distilled water followed by elution with 0.25 N H_2SO_4 . The eluate was collected and its pH was adjusted to 4 by adding 10 per cent sodium bicarbonate solution. The volume was made to 10 c.c. with distilled water and stored in sealed ampoules in frigidaire/deep-freeze for biological assay. Recovery with this method gave satisfactory result in our laboratory.

Assay of catechol amines in the eluate.—As the amount of catechol amines excreted in urine is very small and the calorimetric method of estimation lacks in sensitivity at such concentration, the biological method of assay was adopted. Response produced by given doses of a standard preparation of adrenaline solution in spinal cat was compared to that produced by a quantity of unknown solution and thus the pressure activity of the unknown solution was measured in terms of adrenaline. Total 24-hour content in urine was calculated from the results thus obtained.

RESULTS

In 26 normal adult subjects, 22 males and 4 females, the range of pressor amines was 10.7 to 90.3 microgramme equivalent of adrenaline, the average being 34.8 microgrammes per 24 hours. Standard deviation was ± 21.1 .

The excretion in 24 cases of tropical eosinophilia 20 males and 4 females before treatment was between 8.4 and 70.9 microgrammes equivalent of adrenaline per 24 hours, the mean value being 33.5 ± 16.8 microgrammes. The excretion after the patients completed their treatment, was in the range of 10.2 to 90.0 microgramme equivalent of adrenaline, the mean value being 40.6 ± 22.7 microgrammes.

When these data are subjected to 't' test, it was found that the variations observed amongst the groups—Normal, cases before treatment, and after treatment, were not statistically significant as will be evident from the following Table:—

TABLE

Groups compared	't' values	Remarks
Normal <i>vs</i> before treatment	0.118	Not significant
Normal <i>vs</i> after treatment	0.461	
Before treatment <i>vs</i> after treatment	0.775	

Bronchial asthma.—Only 5 cases has been investigated and catechol amine excretion in 24-hours urine is as below in microgramme equivalent of adrenaline in 24 hours :—

Case No.	Before treatment	After 4 to 6 months symptomatic treatment
1	27.7	29.7
2	99.0	57.1
3	26.0	29.6
4	45.0	..
5	87.4	31.5

It will be evident from above that as in tropical eosinophilia, in bronchial asthma also there is no significant variation in catechol-amine excretion.

EXCRETION OF 17-KETOSTEROIDS IN URINE

Although variations of excretion of 17-ketosteroids, which have got a multiple source of origin, cannot be considered as an adequate indication of altered function of adrenal cortex, as a first step to study the role of adrenal cortex in the pathogenesis of tropical eosinophilia, 17-ketosteroids were estimated in 19 cases and compared with normal values obtained in our laboratory in 21 cases. These 19 cases were also followed up after completion of their treatment. For convenience the male cases within the age group of 20 to 45 years only are included in

this study. Number of female cases are, so far, too small for comparison. Results are as below :—

Group	17-ketosteroids 24-hours urine (mg.)
Normal (21 cases)	7.44 ± 2.48
Tropical eosinophilia before treatment (19 cases)	4.69 ± 1.83
Tropical eosinophilia after treatment (19 cases)	5.97 ± 2.53

Only moderate reduction in excretion of 17-ketosteroids has been observed. Such reduction has also been observed by others in various chronic illnesses and in hospital patients. Returning to normalcy after treatment indicates that the disturbance in 17-ketosteroids is functional in origin.

In 5 cases of bronchial asthma of which 4 were females—results were 4.0, 2.5, 1.5 and 5.9 mg. and one male 6.8 mg. 17-ketosteroids in 24-hours urine.

PAPER ELECTROPHORESIS OF SERUM PROTEIN

Electrophoretic pattern of serum proteins was studied in only 13 cases of tropical eosinophilia and the percentage distribution was analysed with planimeter after properly staining the paper strips. In general, it may be said that there is reduction in the albumin fraction and rise in the globulin of which gamma globulin showed marked rise. In 6 cases, which were studied after treatment, albumin fraction went up over the previous value and gamma globulin fraction showed distinct reduction.

ANIMAL INOCULATION

Oxalated plasma from 13 cases of tropical eosinophilia were injected intraperitoneally into fresh guinea-pig with low initial eosinophilic count. Total w.b.c. and differential counts were made every week and special care was taken to exclude the 'Kurloff' cells. All the guinea-pigs were followed for at least three months and in some cases even up to 6 months. There had been some initial rise in the eosinophil count following injection of plasma but, the rise was not sustained and the rise of count was also not to a marked extent. Moreover, in one guinea-pig injected with plasma from a patient of bronchial asthma, the sustained rise of eosinophil count observed was more than in any other guinea-pig in the previous group, indicating that the rise of eosinophilic count observed may be a non-specific reaction rather than due to a transmissible factor.

In order to investigate further the possibility of a transmissible factor, plasma from the guinea-pig showing marked rise of eosinophil count following plasma injection from the cases of tropical eosinophilia,

was reinjected into other fresh guinea-pigs. Periodic blood count of these guinea-pigs (in 3 cases) did not show any rise in eosinophilic count.

27. Experimental study of the role of adrenal cortex in the genesis of congenital abnormalities under Dr. I.P. Agarwal at the G.R. Medical College, Gwalior.

Fertilized eggs of two strain of hens, i.e. white leg horn (W.L.H.) and Rhode Island Red (R.I.R.) were used for the experimental study. The incidence of spontaneous congenital anomalies was 2 and 26 per cent in W.L.H. in R.I.R., respectively. The comparative susceptibility of fertilized eggs of two types of strains, one with low and the other with high incidence of spontaneously developing congenital abnormalities in chick embryos to various teratogenic agents, differed in both the strains. The following are the preliminary observations :—

1. The injection of 0.0016 mg. of cortisone acetate in the albumin of the fertilized R.I.R. eggs resulted in the induction of various congenital anomalies in the chick embryos. The figures given below clearly bring out the teratogenic action of cortisone acetate :—

R.I.R. hen number	Percentage of spontaneously occurring abnormalities in fertilized eggs in the Control group	Percentage of cortisone induced abnormalities in the fertilized eggs
1	23	55
2	29	All embryos died before 10th day
3	44	-do-
4	14	100
5	0	20
6	25	40

The action of cortisone acetate on the developing chick embryos varied not only with the degree of susceptibility of eggs from different hens, but also on the age of the developing chick embryos at the time of the injection.

2. Various abnormalities occurring in the chick embryos were micromelia club foot, beak deformities, microphthalmia, unilateral or bilateral absence of eyes, dwarfism, ectopia viscerae and exencephaly. These abnormalities were almost similar to those occurring spontaneously and induced with eserine sulphate and insulin.

3. 10 mg. of cortisone was injected into the hens and the fertilized eggs from these hens were collected for $1\frac{1}{2}$ months and incubated as such. The following were the observations :—

PERCENTAGE OF SPONTANEOUS ABNORMALITIES

Hen number	Before injection of cortisone acetate	After injection of 10 mg. of cortisone acetate
1	23	0
3	44	0
6	25	0

From the above data, it appears that cortisone acetate, if injected into the hens, exerts an anti-teratogenic action.

4. The congenital abnormalities in the chick embryos have been successfully induced with eserine sulphate and insulin.

From the preliminary observations it appears that cortisone acetate, the active hormone of the adrenal cortex, can modify morphogenesis and development of the growing chick embryos, depending upon the susceptibility of individual hens, dose of cortisone acetate, and the age of developing chick embryos at the time of injection.

28. Study of the mode of infection and growth characteristics of *rhinosporidium seeberi* under Dr. Indu Mohan Gupta at the Kasturba Medical College, Mangalore.

Two cases of rhinosporidial growth, both from nose, came under investigation during the period April, 1957 to September, 1957. Specimens removed at operation were collected in sterile physiological saline. One part of the growth was subjected to histopathological study. From the remaining portion, attempt was made to grow the causative organism on different artificial culture media and also tissue transplants of the growth were made aseptically into the buccal mucosa of the guinea-pigs. Attempts to grow the organism have so far not been successful.

During the course of cultural study it was interestingly observed that the wall of the sporangium of *rhinosporidium seeberi* is markedly resistant to prolonging desiccation. The resistant nature of the wall of the sporangium in general, may presumably be related in some way to the failure in obtaining the rhinosporidial growth on artificial culture media and in tissue transplant experiments.

29. Inquiry to find out a suitable medium and method for culturing rhinosporidiosis which had not been cultured so far under Dr. H.S. Andleigh at the S.M.S. Medical College, Jaipur.

It was thought that *Rhinosporidium seeberi* could grow either on special media or in the living tissue of the animals, such as monkeys and rabbits. For these purposes a small tissue was excised from the *Rhinosporidial* polyp in the nostril of the patient. It was then immediately, but gently, broken up in brain-heart-infusion liquid medium in a sterile glass pestle and mortar.

A part of the suspension was inoculated on specialized media containing human blood and growth factors, such as yeast, cystine and Biotin. A part of the suspension was then injected into the mucous membrane of the nostril of the monkey and the rabbit. During this period four cases of *Rhinosporidiosis* attended the Hospital but I could get material from three cases only and this was injected into the nasal mucosa of the monkey and the rabbit.

All attempts to grow the organism on artificial medium containing human blood and growth factors, such as yeast cystine and Bietin did not yield any growth of *Rhinosporidium seeberi*.

No growth or abnormality has appeared as yet in the nasal mucous membrane of either the monkey or the rabbit. They are being kept for further observation.

RESULTS

1. All attempts to grow the organism on special media have failed.
2. No growth has yet appeared in the nasal mucosa of the monkey and the rabbits inoculated with a suspension of the excised polyps.

30. Investigation of antistreptolysin titres in children under Dr. S.L. Bhatia at the Lady Hardinge Medical College, New Delhi.

Attempts at preparing *Streptolysin* 0 in the laboratory were not successful. The group A *Streptococcus* obtained from Colindale (National Collection of Type Cultures) was found not to be viable and the strains isolated from cases investigated in the laboratory were used and were not satisfactory.

A small amount of *Streptolysin* 0 was obtained from U.S.A. A certain number of sera were put up and the results were as follows:—

Normal cases of children with no history of recent sore throat.

Case No.	Antistreptolysin titre
N. 1	80
N. 2	60
N. 3	80
N. 4	20
N. 5	0
N. 6	100
N. 7	100

*Active rheumatic cases—majority from
Himachal Pradesh obtained by
Dr. Devi Chand*

A.S.O. titre.

Case No.	R.		1,000	Units.
	R.	1	100	„
	R.	2	1,000	„
	R.	3	2,000	„
	R.	4	4,000	„
	R.	5	1,000	„
	R.	6	1,000	„
	R.	7	1,000	„
	R.	8	1,000	„
	R.	9	1,000	„
	R.	10	600	„
	R.	11	2,000	„
	R.	12	4,000	„
	R.	13	4,000	„
	R.	14	4,000	„
	R.	15	800	„
	R.	16	2,000	„
	R.	17	4,000	„
	R.	18	2,000	„
	R.	19	2,000	„
	R.	20	800	„
	R.	21	1,000	„
	R.	22	100	„
	R.	23	1,000	„
	R.	24	1,000	„
	R.	25	4,000	„
	R.	26	2,000	„
	R.	27	80	„
	R.	28	1,000	„

A large number of sera have been collected and await investigation.

31. Comparative study of serological techniques in detecting antibodies following immunization with *S. Typhi* 0 antigen in rabbits and sera of patients suffering from enteric fevers under Dr. A.K. Banerjee at the S.S.K. Memorial Hospital, Calcutta.

Preliminary work on the immuno-chemistry of the specific polysaccharide hapten of the 0 antigen of the smooth form of *S. typhi*

is in progress. A non-motile 0 variant of smooth form of *S. typhi* was used in the investigation. A method has been standardized for preparing an aqueous extract of the 0 antigen of *S. typhi*. The specific polysaccharide was obtained by acetone treatment of the quantitative yield of polysaccharide per gram of bacteria was determined. The effect of different cultural condition and the effect of duration of growth on the yield of specific polysaccharide is being studied.

32. Inquiry entitled 'Early diagnosis of enteric fevers' under Dr. N. P. Gupta and Dr. S. P. Gupta, at the Medical College, Lucknow.

(1) Experiments were carried out to determine the presence of soluble polysaccharide antigen of *S. typhi* :—

- (a) In the urine of patients of typhoid fever.
- (b) In the sera of patients of typhoid fever.
- (c) In the sera of mice inoculated with *S. typhi murium*.
- (d) In the urine and fæces of mice inoculated with *S. typhi murium*.

Chicken red cells were sensitized with cell free extracts of *S. typhi* and *S. typhimurium* as required for different tests. Those sensitized red cells were used as agglutinable suspensions. A standard '0' serum of known titer was put up in a standard volume in doubling dilutions. In a parallel titration a suitable volume of urine, serum or fæcal extract was added. The titre of the standard serum in the presence of hypothetical soluble antigen was noted and recorded.

The hæmagglutination inhibition tests were carried out with the urine and sera of typhoid patients and urine, sera and fæcal extracts of mice (in which typhoid was produced experimentally), to detect the presence of soluble antigen.

Urine of 8 patients of typhoid fever with a positive blood culture was investigated, and the results showed that there was some indication of presence of soluble antigen in 5 out of 8 cases.

Hæmagglutination inhibition tests were carried out with sera of 16 patients in which blood culture was positive. The results indicated that only in 3 cases there is some indication of presence of soluble antigen.

(2) The technique of production of experimental mouse typhoid has been standardized, and viable counts carried out to determine the phases of bacteræmia. Mice were kept in metabolic cages so that their urine and fæces could be collected easily. Preliminary tests have been carried out to determine, if urine, fæcal extract and serum from mice with maximal bacteræmia would cause inhibition of hæmagglutination test with standard serum prepared against 0-antigens of *S. typhi murium*.

33. Inquiry into the problem of chronic splenomegaly and its relation to hepatic pathology under Dr. B. K. Aikat, and Dr. A. K. Basu at the Institute of Post-Graduate Medical Education and Research, Calcutta.

The work was carried out according to the approved plan of study.

(1) *Clinico-pathological study*.—A total of 31 cases of chronic splenomegaly have so far been investigated in detail. Twenty-nine of these cases have been operated on and are being followed up.

RESULTS.

The data obtained are summarized as follows:—

Number of cases

(1) *Pre-operative :*

(a) *Clinical :*

Significant history of fever	...	...	9
Haemorrhage-hæmatemesis and melaena	...	...	16
Jaundice	...	...	1
Pallor	...	...	31

(b) *Heamatological :*

Anæmia	...	...	31
Normocytic normochromic	...	...	19
Microcytic hypochromic	...	...	12
Leucopenia (below 3,000/cmm.)	...	...	20
Thrombocytopenia (below 100,000/cmm.)	...	...	7

(c) *Bone-marrow study :*

Hyper-cellular marrow	...	...	29
Normoblastic	...	...	29
Hypo-cellular normoblastic	...	...	2

(d) *Liver function studies*.—A battery of liver function tests were performed which included total proteins, serum albumin and globulin by micro kjeldahl method, electro-phoretic study of serum protein by 'moving boundary technique' thymol, zinc sulphate turbidity tests, cephalin cholesterol flocculation tests, total serum bilirubin and alkaline phosphatase.

On the above basis liver function was considered :—

Normal	...	...	<i>No. of cases</i> 8
Moderately damaged	...	...	9
Severally damaged	...	...	14

(e) *Radiology :*

(a) Ba swallow	...	...	14
Oesophageal varix	...	...	

(b) *Splenoportal venography :*

(i)	Extra hepatic obstruction	...	9
(ii)	Dilated tortuous splenic vein without collaterals		6
(iii)	Dilated tortuous splenic vein with collaterals		16

(2) *Operative study.*(a) *Portal venous pressure :*

(i)	Below 200 mm saline		2
(ii)	200-300 mm saline		10
(iii)	Above 300 mm saline		15

(b) *Appearance of liver :*

	Coarsely nodular		14
	Diffuse granularity		4
	Wide scarring		2
	Atrophy of left lobe		2
	Fatty liver		2
	Enlarged without granularity		2
	Normal appearance		5

(c) *Presence of thrill in splenic artery* ... 6*Operative procedure :*

	Splenectomy alone		16
	Splenectomy with splenorenalshunt		9
	Porta-caval shunt		1
	Ligation of hepatic artery		3

Final aetiological assessment of cases.(a) *Cirrhosis of liver with portal hypertension and fibro-congestive splenomegaly* ... 20(b) *Extrahepatic hepatic obstruction with portal hypertension and fibrocongestive splenomegaly* 9

(i)	Portal venous thrombus	..	1
(ii)	Cavernomatous transformation of portal veins		3
(iii)	Chronic pancreatitis		1
(iv)	Pancreatic tumour		3
(v)	Hypertrophic candate lobe pressing on portal vein		1

(c) Hyperplasia of the Reticulo endothelial cells of the spleen.

			No. of cases.
(i)	Kala-azar	...	1
(ii)	Unknown...? Malaria	...	1

Histology of the liver :

Post necrotic cirrhosis	...	...	14
Diffuse hepatic fibrosis	...	...	4
Post necrotic scarring	...	...	2
Protein malnutrition	...	...	2
Fatty liver	...	...	5
R.E. hyperplasia	...	...	3
Normal structure	...	...	8

Histology of the spleen :

Fibro congestive-splenomegaly	...	...	24
Primary R.E. endothelial-cells hyperplasia	...	...	3
			including 1 K.A.
R.E. hyperplasia in association with fibro-congestive splenomegaly	...	...	24
Myeloid Metaplasia	...	...	11
Infarcts	—	...	7

Follow up study.—This consists of periodical and regular clinical, hæmatological, radiological examination of the patient combined with evaluation of the structural and functional status of the liver.

Experimental work in animals.

(1)	Monkeys :		No. of cases.
	Supra and infra hepatic obstruction— Mechanical with cellophanetape :		
	Supra hepatic obstruction	...	6
	Infra hepatic obstruction	...	4
(2)	Infra hepatic obstruction :		
	Production of cirrhosis in Albino rats by injection of 0.1 ml. of carbon tetrachloride per 100 gm. of body wt. once a week	...	36
(3)	Splenic hyperplasia :—		
	Biweekly injection of 0.2 cc. per 100 g. of wt. of 2.5 per cent methyl cellulose of viscosity grade of 400 centipoises.		
	Normal diet and methyl cellulose	...	18
	Low protein diet (2 per cent protein) and Methyl cellulose	...	18
	Low protein diet alone	...	18

The data from the experimental work cannot be analysed at the present stage.

There is good deal of suggestive evidence that there is marked increase in the splenic blood volume and outflow in most of the cases with presence of a distinct thrill in the splenic artery in some cases.

34. Inquiry into electrophoretic study of immunised rabbits with particular reference to immunological tolerance under Dr. D. Barua at the S.S.K. Memorial Hospital, Calcutta.

The term immunological tolerance refers to a specific weakening or suppression of re-activity caused by the exposure of animals to antigenic stimuli before the maturation of the faculty of immunological response. Rabbits have been selected as the experimental animal and *S. typhi* as the antigen to see whether these animals develop immunological tolerance to this bacterial antigen when challenged in their foetal or early life. The experiments so far carried out are described under five headings :

(a) Four adult rabbits of about four months of age were inoculated by 3 intramuscular injections of 50⁶, 100⁶ and 200⁶ of heat killed *S. typhi* at five days intervals. The titre obtained were varying from 1:5120 to 1:20480 against TH and TO antigens. Electrophoretic study was done by 'boundary zone' method in an Antweiler apparatus and showed high peaks of gamma-globulin. Absorbed sera with homologous antigen showed fall of the gamma-globulin peak.

(b) Electrophoretic pattern of sera of five adult and 10 young unimmunized rabbits was studied by the same method. The electrophoretic globulin pattern of one and two days old rabbits was found to be different from that of adult rabbits.

(c) Two pregnant rabbits were immunised with *S. typhi* antigen by the same method as was employed in the experiment A commencing from the 15th day of gestation. The sera of their 7 baby-rabbits (3 from one litter and 4 from another) were found to contain antibodies against *S. typhi* and the titre was found to be between 160 and 1:320. When they were 3 months old the titre of their sera fell to 1:20 to 1:80. Out of these 7 litters 2 died. Remaining 5 litters were available for subsequent immunization when they were of 4 months age. The schedule of immunization was same as in their mothers. Two of them showed rise of titre to 1:160 and in 3 others the titre went upto 1:320.

(d) Two embryos were injected intraperitoneally in utero on the 30th day of gestation with about 25⁶ organism. One of them died after birth. The other one did not develop any antibody when tested 6 weeks after birth. This animal was immunized by the 3 injection schedule against *S. typhi* and the titre did not go above 1:320.

(e) Out of 4 litters (13 rabbits in all), 9 were injected intramuscularly with 25⁶ organism within 1 to 16 hours of their birth and one from each of the 4 litters were kept as control without any immunizing

dose. Four of the 9 test animals died within 3 or 4 days. Remaining 5 test animals and 4 control animals did not show any antibody when their sera were tested with *S. typhi* antigen 6 weeks after birth. All the 6 animals (5 test animals and 4 control animals) were then immunized with the 3 injection schedule. All control animals (four) developed antibodies, the titre varying from 1:2560 to 1:5120. In the test animals (five), the titre varied from 1:160 to 1:320. It is not possible to try to draw any conclusion about the state of immunological tolerance against bacterial antigens in rabbits from the experiments that have so far been done. Attempts are being made to increase the number of animals in each experiment and to evolve new experiment to prove or disprove this.

35. Study of kwashiorkor with special reference to histopathological and histochemical changes in the cutaneous lesions under Dr. C. Mohan Rangan at the M.G.M. Medical College, Indore.

The material studied so far consists of skin biopsies from 8 children with typical cutaneous lesions of kwashiorkor and 4 normal healthy skins. The criteria for selection of cases were the presence of œdema, retarded or arrested physical growth, mental apathy, enlargement of liver, hypoalbuminæmia, and typical cutaneous lesions, i.e. crazy-pavement dermatosis, enamel paint dermatosis, parchment dermatosis and crackled skin appearance. In all but two cases, biopsies were taken from the skin of the abdominal wall. In the other two cases, biopsies were taken from the lateral aspect of the leg.

The sections from the formal saline processed tissue were stained with hæmatoxylin and eosin for routine study. The collagen, elastic tissue and melanin were studied by Van Gieson, Verhoff and Fontana stains, respectively. The sections from alcohol-fixed tissue were stained with congo red hæmatoxylin for keratohyaline and eleidin.

It has been observed by previous workers that whilst pellagrous lesions occur in regions exposed to sunlight, kwashiorkor lesions seldom do so. We are unable to confirm this observation since in our series the lesions have been observed over the dorsum of hand and forearm and extensor surfaces of the legs, distribution regarded as typical of pellagra.

Histologically, changes have been observed in the epidermis and dermis. In the epidermis, the most striking feature was atrophy of which the following patterns could be recognized.

Epidermis.—

- (a) Marked and uniform atrophy with no hyperkeratosis or parakeratosis.
- (b) Irregular epithelial atrophy alternating with normal stretches causing a marked corrugation of surface.
- (c) Epithelial atrophy with co-incidental hyperkeratosis and parakeratosis.

In areas showing marked atrophy, the stratum malphigi and stratum basalis are so greatly decreased in thickness that the entire thickness of the epidermis seems to be constituted by strata granulosa and lucida. In the atrophic areas the stratum granulosum is depleted of keratohyaline granules.

The disturbances in the basal layer seen maximally in the atrophic areas but elsewhere also consist in an apparent displacement of these cells and loss of cohesion between them. The melanin pigment is absent in the basal layer and is dispersed in the stratum malphigi and also in dermis. In the latter case the pigment is found lying either loose or held by histiocytes.

Dermis.—The fine wavy collagen of the papillary layer shows an œdematous thickening, separation of the fibrils and fragmentation. The thicker bundles of collagen found in the sub-papillary layer also show similar features. The elastic fibres show fragmentation and clumping. There is generalized infiltration of the dermis with histiocytes best seen in the papillary layer.

Even at this stage the cutaneous lesions observed in kwashiorkor seem to be attributable to multiple deficiencies. The hypoproteinemia observed by us would explain the œdema in the dermis. Perhaps, the increased permeability of the dermal capillaries due to deficiency of vitamin C reported by previous workers would aid and aggravate the development of œdema. The latter appears to be responsible for fragmentation of collagen and elastic tissue.

The epithelial atrophy is the result of dermal œdema and the consequent compression of the dermal capillaries feeding the epidermis. The hyperkeratosis and parakeratosis is explainable on the basis of vitamin A deficiency and perhaps vitamin B complex deficiency. Keratohyalin granules, being protein in nature, are depleted in kwashiorkor, a disease of protein deficiency.

Further, it seems possible to correlate the clinical appearance of any particular type of lesion with the specific histopathological changes, e.g. in parchment dermatosis there is uniform and marked epithelial atrophy, in crazy-pavement dermatosis or enamel paint dermatosis, there is combination of hyperkeratosis and parakeratosis with atrophy, and in crackled skin appearance there is marked corrugation of the epidermis. The simultaneous incidence of atrophy and hyperplasia has not been reported in pellagra.

36. Studies on the role of inositol in hyperlipemic conditions under Dr. V. Srinivasan at the Madurai Medical College, Madurai.

The influence of inositol on lipid metabolism was found to be associated more with the cholesterol metabolism than with the other lipid fractions. Hexa-chlorocyclohexane (Gammexane), the anti-metabolite for inositol was found to inhibit the cholesterol esterase activity of liver and pancreatic extracts. This inhibition was found to be reversed almost completely by inositol.

- 37. Study on the effects of selected respiratory stimulants in states of pulmonary insufficiency associated with hypercapnia and hypoxia under Dr. N.R. Konar at the Nilratan Sircar Medical College, Calcutta.**

Ten cases of pulmonary insufficiency were taken. In addition to a detailed clinical survey, respiratory function tests, including spirometry and arterial blood gas analysis, were done in them before and after injection of 0.24 g. of aminophylline slowly by intravenous route.

Oximeter was calibrated against van Slyke manometric apparatus.

Clinically, all the cases showed signs of emphysema and after the injection of aminophylline, patients became more restful and dyspnoea was relieved to some extent. Respiratory rate was decreased in 7 cases and increased in 3 cases, but the depth of respiration was increased in all the cases. Pulse rate slightly decreased in all the cases. Slight cyanosis was present in 2 cases before injection and it disappeared after injection. Rhonchi was much less after injection.

Regarding respiratory function tests, injection of aminophylline stimulated respiration; particularly the amplitude of respiration was increased, and in all the cases there was a net increase of pulmonary ventilation. The vital capacity and maximum breathing capacity were also increased. The percentage of total vital capacity in 1st and 2nd seconds was increased in all the cases investigated. Ventilatory equivalent of oxygen, however, increased in all cases.

Oxygen content and percentage saturation of arterial blood were increased moderately. The carbon dioxide level of arterial blood showed a change in the reverse direction (In one case, injection of aminophylline was followed by 31 per cent increase in pulmonary ventilation, 37 per cent increase in oxygen consumption per minute and about 70 per cent increase in oxygen content of arterial blood. The calculated oxygen tension of arterial blood increased from 66 to 103 mm. of Hg.; there was, however, no significant alteration in the carbon dioxide figures in this case).

- 38. Inquiry entitled, 'Pollination Calender for Greater Delhi' at the Vallabhbhai Patel Chest Institute, University of Delhi, Delhi.**

A provisional list of the allergenic plants of Delhi along with the chronology of their pollination season has been compiled. An herbarium and a reference collection of permanent pollen slides of allergenic plants which flowered during the past four months (since June 1957) have been prepared. The incidence of atmospheric pollens at three stations selected in different parts of the city has been recorded. Collection of large amount of pollens from suspected allergenic plants for the extraction of antigens is being carried on.

39. Inquiry on the relative values of colpomicroscopy and vaginal cytology in the detection of early carcinoma of cervix uteri under Dr. Chinmoy Ghose at the Calcutta National Medical Institute, Calcutta.

The detection of early carcinoma of the cervix uteri is of paramount importance to achieve maximum salvage of patients with modern armamentarium at our disposal. The conception of what constitutes the early stage has changed radically during recent years. Carcinoma-in-situ, intraepithelial or pre-invasive carcinoma before the basement membrane barrier is broken has been widely accepted. Unfortunately cervixes with pre-invasive carcinoma seldom arouses any suspicion on clinical examination. Cervixes that present detectable clinical lesion, diagnosis is usually established on tissue biopsy.

Cyto-diagnosis.—Cancer cells being exfoliative in nature, detection of these cells in the vaginal discharge has been universally employed because of its simplicity and wide scope of screening the total population of women to detect the incipient carcinoma. There are obvious fallacies with a cyto-negative or cyto-positive diagnosis.

Colpo-microscopy.—Hinselmann's (1924) original devise of Colposcope gives a magnified view (10-40 times) of the cervix and has been found to be useful in selecting sites for biopsy. A great improvement over this magnification has been incorporated in the recent invention of Colpo-microscope (Antoine and Grunberger) which gives a magnification of 110-280 times. Colpo-microscopy therefore offers great scope of microscopic scanning of the stained living epithelium of the portio vaginalis of the cervix in an ambulatory patient during routine gynaecological examination. The method is simple and once the unit is set up and one gets used to such examination, the microscopic scanning of the whole cervix does not take more than 10 minutes. The other advantages are, (1) sterile working is not necessary, (2) examination can be repeated as often as is necessary, is harmless and causes no pain, (3) the superficial epithelial layers or modified areas at the surface and slightly deeper seated cell layers upto a depth of about 0.7 mm. below the surface could be seen just one would see a stained histological slide under an ordinary microscope. There are, however, some disadvantages too. The present study is aimed to find a co-relation between colpomicroscopy and vaginal cyto-diagnosis supported by tissue biopsy whenever indicated in the detection of incipient or early carcinomatous lesion of cervix.

Unselected patients of 25 years of age and over attending the out patient's department are subjected for study. Cytological smears for papanicolaou stain are made from (a) vaginal discharge collected from posterior fornix, (b) surface scraping by Ayre's spatula from Squamocolumnar junction. A hanging drop preparation is made to exclude *Trichomonas* infection. History, other gynaecological findings with special note of the clinical condition of the cervix are recorded. Colpo-microscopy is done on the same day or at a subsequent date. When indicated cervical biopsy is taken from the areas selected on naked eye

or Colpo-microscopic findings. 8 to 36 histological sections are prepared for study. Cytological and Colpo-microscopic examination are repeated when necessary.

During a period of three months total number of patients examined were 217, of these, only cytology was done in 58 cases, cytology and Colpo-microscopy in 156 cases and cytology, Colpo-microscopy and biopsy in 24 cases. The cases may be grouped as follows :—

1. Cervix clinically healthy 130 cases.
 - (a) Colpo-microscopy (C.M). } Negative in 71 cases.
Cytology
 - (b) Only Cytology done Negative in 58 cases.
(Patients failed to report for
C.M. examination).
 - (c) C.M.—Negative } In 1 case.
Cytology—Positive
 - (d) C.M. } Both positive—no case
Cytology. } encountered.
2. Cervix clinically unhealthy but did not appear malignant 82 cases.
 - (a) C. M. } Both negative in 79 cases.
Cytology.
 - (b) C. M. } Both suspicious in 3 cases.
Cytology.
3. Cervical biopsy-negative in 2 cases by serial sections. In one case, biopsy showed moderate basal cell activity in one area. Examination of whole cervix by serial sections after total Hysterectomy did not reveal any activity in other areas. The cases are under follow up.
3. Advanced clinical carcinoma of cervix in 3 cases.
C.M. could not be done due to hæmorrhage.
Cytology—Positive in 2 cases.
Biopsy—Invasive carcinoma in all 3 cases.
4. Vulval Carcinoma—1 case.
C.M., Surface scraping smear and Biopsy were all positive.
5. Vaginal vault recurrence—1 case.
C.M.—Positive.
Cytology—Negative.
Biopsy—Positive.

The patient was followed up post-operatively after radical abdominal hysterectomy for carcinoma cervix with pregnancy. Cyto-diagnosis was twice negative but colpomicroscopy revealed typical malignant cells at the shallow vaginal vault, confirmed by subsequent biopsy. The study is under progress no conclusion can be drawn at this stage.

40. Studies to investigate the role of mucopoly saccharides in the formation of kidney stones under Dr. K.N. Udupa at the Himachal Pradesh Hospital, Simla.

For this research scheme, the blood, urine and kidney biopsies of the normal people and those suffering from kidney stones were studied biochemically and histochemically with regard to the mucopolysaccharide content and other related substances. The methods of the study and the results obtained are briefly described below.

1. *Blood*.—Ten healthy members of the staff of the Himachal Pradesh Hospital were selected as the control subjects for this study. The serum mucopolysaccharides as determined by the glucosamine content varied from 62.5 mg. to 92.5 mg. the average being 72.5 mg. per 100 cc. In the same persons the serum calcium and phosphorous contents were also estimated to correlate the same with that of mucopolysaccharides. The serum calcium varied from 8.0 mg. to 14.0 mg. the average being 11.0 mg. per 100 cc. The phosphorous content varied from 3.0 to 5.0 mgm. the average being 3.75 mg. per 100 cc.

Ten patients suffering from various types of kidney stones were also studied for the above constituents and found that serum mucopolysaccharide contents of the patients varied from 58.75 to 82.5 mg. the average being 72.5 mg. per 100 cc. The serum calcium of these patients varied from 8.6 to 14.4 mg. the average being 11.0 mg. per 100 cc. The serum phosphorous varied from 3.0 to 5.0 mg. the average being 4.0 mg. per 100 cc. From these preliminary studies it is at once clear that there is no significant difference between the normal and those patients with renal lithiasis so far as the blood mucopolysaccharides, calcium and phosphorous are concerned.

2. *Urine*.—All the studies in the urine were carried out in the 24 hour urine specimen. These were analysed for glucosamine contents as per the same methods with certain modifications as was done for the serum. The urine calcium and urine phosphorus excretion rates were also determined and calculated for the 24 hour period. The results of this study indicate that in normal persons the mucopolysaccharides, calcium and phosphorous excretion rates were 154.2 mg./24 hrs., 650 mg./24 hrs and 1200.0 mg./24 hrs. But in those cases with kidney stones, the findings were 72 mg. and 6024 mg. and 336 mg. per 24 hours respectively. From these findings one can conclude that patients with renal calculi excrete lesser quantities of mucopolysaccharides and phosphorous and large amounts of calcium. This has been a consistent finding in all our cases so far studied. However, further studies in more cases will have to be done to arrive at a definite conclusion.

Histochemical studies of the kidney biopsy.—Kidney biopsies were taken from normal persons and from those suffering from kidney stones. Normal kidney biopsies were made when we were operating in the neighbourhood of the kidney and a few fresh autopsy specimens were also taken for these studies. The biopsies of the kidneys with stones were invariably made during operation. All the specimens were studied histologically and histochemically. From preliminary observations it appeared that in the kidneys with stones there is derangement of mucopolysaccharides both quantitatively and qualitatively in distribution especially in and around the urinary tubules.

From these findings it seemed that there is some retention of mucopolysaccharides in the kidneys which might be responsible for the initiation of nidus formation for the stones in the kidney. Their future growth and development into typical stones might take place in the calyces where the decreased mucopolysaccharides and increased calcium content of the urine might further contribute to their development.

41. Induction of gall stones in monkeys under Dr. B.N. Balakrishna Rao at the G.R. Medical College, Gwalior.

The problem of gall stones is the problem of calculus formation, in general, and cannot yet be said to have been solved. In this Laboratory, urinary stones have been successfully produced by introduction of F.B. nucleus in the urinary bladder of dogs (T.B. Singh *et al*, 1954). On a parallel study, this work was undertaken to elucidate the role of obstruction, infection and implantation of nuclei in the production of gall stones in monkeys.

Three types of nuclei (zinc, magnesium and pieces of human gall stones) have been used as intracholecystic implants. Serial studies of blood cholesterol, roentgenograms, pH of bile and histological examination of gall bladder wall are being carried out. The effect of cystic duct obstruction after nucleus implantation is also being observed.

Till the time of this report, stones have not formed. In most cases monkey bile was found slightly on the acid side and this factor might be responsible for the prevention of deposition of crystalloids (e.g. calcium carbonate).

With the idea to possess an all-time easy access of gall bladder bile for serial examination and wilful manipulation, a polythelene tube from the inside of the gall bladder to the outside on the surface of the abdominal skin is under trial in dogs. It would be worthwhile to observe the effect of controlled changes in bile pH.

42. Effect of tissue therapy in the prevention of blindness due to degenerations and abiotrophies of the retina and choroid under Dr. J. Bose at the R.G. Kar Medical College, Calcutta.

Altogether we examined 22 cases of pigmentary degenerations of retina, out of which 10 cases are primary and 12 cases are secondary. Of

all the cases examined by us, only one case was female who is a secondary one and rest are males. The age of the patients, examined by us, varied from 6 years to 44 years and the age of onset of the night blindness, (as told by the patient, or its attendant) varied from 3 years to 12 years. From the genetical study, we have found that in 2 cases there is dominant heredity and in one of them *Retinitis pigmentosa* was present in the same generation. The visual acuity was examined in the modified Landolt's ring charts.

All the cases were examined clinically and investigated properly and from the investigations and clinical studies we divided the cases into primary and secondary. Out of 12 secondary cases, only one was a female. Age variation was between 13 and 46 years, while the age of onset, as obtained from the history, was between 9 and 44 years. While investigating the ætiology, it was found that 5 cases showed positive Mantoux test over 1 in 10,000 dilutions (tubercular).

Only 3 cases showed a positive Wassermann and Kahn's precipitation test (syphilitic), while in 4 cases, ætiology could not be determined. However, on clinical basis, these undetermined groups could not be included into the primary series as these patients showed a normal dark-adaptation and had no night blindness. They had, on the contrary, certain amount of day blindness. The perimetric observations were done in all the cases and the records of the observations were noted. The dark adaptation test was done in most cases (both primary and secondary) by means of Della Casa adaptometer.

Preparation of aqueous extract of placenta.—The placenta (Caesarean or normal delivery) was kept in the refrigerator for 8 to 10 days in the same temperature, mentioned below. The refrigerated placenta was washed thoroughly with distilled water to remove the blood and mucous. Then 25 g. weight of placenta was cut from the depth of the different cotyledons and then minced very thoroughly. The minced placenta was finally triturated in a mortar. 250 c.c. of sterile normal saline was then added and then kept at room temperature for one hour with occasional stirring. After that the solution was heated in a water bath (temp. 60° C.) for one hour. The solution was then passed through cotton and paper filters. The filtrate was again filtered with paper filters several times but as the sediments were still left, the filtrate was finally passed through glass-wool filter. The filtrate was then boiled in a water bath (temp. 60° C.) for 15 minutes, and then sealed in glass ampoules. The sealed ampoules were then autoclaved for 45 minutes (temp. 118°C. and 15 lb weight). The autoclaved ampoules containing the solution were then kept in a refrigerator again (temp. 2°C. to 4°C.). The protein content of the placenta was then examined and finally aerobic and anærobic culture examination was done before injecting to patients. Before injecting the solution to human beings it was injected to a guinea-pig to see any reaction. The sealed ampoules were always kept in refrigerator. Unfortunately, turbidity and reddish colour of the filtrate could not be driven out. We are still investigating about the causes of these defects specially the higher percentage of protein than Filatov's specification (.01 percent).

TECHNIQUE OF TISSUE THERAPY

(A) *Grafting*.—At first, the grafting was done in the subtenon's space (*vide* comprehensive report submitted in 1956). Next the subconjunctival spaces were tried but due to extreme fibrosis the technique was modified and implantation done in the subcutaneous space of the anterior abdominal wall. Later, this was changed to the proximal portion of the ventral aspect of the forearm. When the implanted tissue was bigger than the size of a pea the stitches gave way due to tension and the implanted tissue came out and as such we implanted less placental tissue and made the surgical wound bigger.

The placenta grafting was always done under local anæsthesia and under strict aseptic measures. In the subconjunctival and subtenon's route there was sometimes pain after the action of the local anæsthesia was over and the congestion used to last for more than 5/6 days but there was no infection and coming out of the tissue except once or twice. But in the subcutaneous route, there was no pain during and after operation excepting one who felt too much pain after the operation but there was no infection. There was no stitch abscess except in only one case.

We usually graft 4 times in a year at an interval of 3 months.

(B) *Injection of aqueous extract of placenta*.—The aqueous extract of placenta (so far of Albert David & Co. Ltd.) was injected by intramuscular route daily. Forty five such injections were done at a stretch during which one implantation was done along with the injections of aqueous extract. Four such courses were done in a year. The ampoules were always kept in a refrigerator at a temp. of 2°C. to 4°C. Sometimes patients were supplied ampoules with an advice to keep them in ice flask. There are no untoward symptoms with the injections of the aqueous extract of placenta upto this day.

43. Inquiry into blood stream cooling as a method of inducing and maintaining hypothermia under Dr. A.K. Basu at the S.S.K.M. Hospital, Calcutta.

Thirty-five animal experiments have been carried out during the period from May to December 1957. The experiments have been divided into 3 categories.

1. STANDARDIZATION OF TECHNIQUE OF EXTRA-CORPOREAL VASCULAR COOLING FOR HYPOTHERMIA AND STUDY OF THIS METHOD OF COOLING AS CONTRASTED WITH THE SURFACE METHOD.

Twenty-six experiments were carried out under this category. The technique employed in per vascular cooling was as follows :—

- (a) Cannulation of femoral artery and vein by polythene cannula.
- (b) Circulation of the blood by means of a hand pump through a latex tube submerged in brine mixture (average temp. — 4°C).

- (c) Heparinization of the animal's blood to prevent intravascular clotting.
- (d) Electro-cardiographic hook up.

All these experiments were carried out with the animal under I.V. pentothal anæsthesia with endotracheal intubation.

In addition, in 2 experiments, lytic cocktail consisting of pethidine, phenergan and chlorpromazine was used.

A number of difficulties were met with during the carrying out of this experiment. These included :

- (a) Thrombosis of blood in the cooling system.
- (b) Air embolism.
- (c) Deficient cooling due to insufficient cardiac action in the absence of a pump in the system.
- (d) Sudden ventricular failure due to loss of peripheral resistance.

These were gradually overcome as experience was gained in the experiments.

It was found that the per vascular method of cooling was generally slower than surface method of cooling and entailed a number of difficult technical procedures which were sometimes associated with complications.

2. UTILIZATION OF TECHNIQUE OF HYPOTHERMIA FOR CREATION AND CORRECTION OF INTRACARDIAC DEFECTS.

Method.—Under surface cooling, the temperature was lowered to 28°C. Right-sided thoracotomy exposed the superior and inferior vena cava which were controlled with loops. The azygos vein was divided.

After pericardiotomy, the cardiac inflow was occluded and the right atrium opened. The interatrial septum was exposed, and a standard size defect created. The atrial wall was closed and the cardiac inflow was re-established by releasing the loops. The average time of cardiac inflow arrest was 7 to 9 minutes.

Three animals survived the procedure and 6 died. No death was due to ventricular fibrillation or attributable to the direct effects of hypothermia.

3. Biochemical and histological studies of different types were undertaken. Blood sugar, blood urea, blood uric acid, liver glycogen, and myocardial potassium were estimated in a number of experiments. There was a general tendency towards rise of blood sugar and fall of liver glycogen and of myocardial potassium. Blood urea and uric acid did not show any significant variation.

Histological studies were made in a number of experiments of different parenchyma, such as liver, lungs, myocardium, and kidney. No definite information can be given at present.

44. Inquiry under investigation into the pathogenesis of spinal concussion following injuries of spine under Dr. H.K. Sarkar at the S.S.K.M. Hospital, Calcutta.

The following methods were tried to produce spinal concussion in rabbits :—

1. The dura in the dorsal region was exposed by laminectomy under local anæsthesia (1 per cent novocain 7 c.c. to 10 c.c.). The cord enclosed in the dura was traumatized by the tip of an artery forceps. Six animals were used.

2. Acute forcible flexion of neck was attempted under x-ray screening in another 6 animals. It became apparent that it is almost impossible to dislocate or fracture a cervical vertebra.

3. This was done in 2 stages.

Stage I.—The idea of this stage is to impair the stability of the cervical spine. This was done under local anæsthesia by separating the posterior cervical muscles from the spines and laminæ and dividing the ligamentum nuchæ, inter-spinous and inter-laminar ligaments. The wound was closed by apposing the post cervical muscles over the damaged spine by interrupted catgut stitches. The rest of the wound was closed by continuous catgut. During the operation, 5 of the 15 animals showed transient paresis of the hind limbs for 1 to 4 hours.

Stage II.—This was done after 8 to 10 days of the first operation when the external wound was completely healed up. The animals were acutely flexed at the neck just caudal to the site of injury under x-ray control. In 3 out of 15 animals, there was complete paralysis of the hind limbs with paresis of the fore limbs, whereas in the rest paresis of only the hind limbs was produced. These animals were observed to lie on the side of the trunk with no effort to move. Feeding had to be helped. After 48 hours the limbs became spastic. Four to six days after operation the animals were observed to stand on their legs if supported. In another 8 to 10 days time the animals behaved normally. By this method it became possible to produce in animals post-traumatic complete or partial paralysis of the limbs with subsequent full functional recovery within 2 weeks' time. This condition is akin to the phenomenon of spinal concussion in human beings observed following injury of the cervical spine.

45. Hydrocephalus in infants and children, investigation of its etiology and treatment under Dr. R. Nigam at the Medical College, Nagpur.

This report embodies the work done during the period ending 30th September, 1957. The work consists of two parts.

(a) *Experimental production of hydrocephalus.*—Two common laboratory animals, the albino rat and the dog, have been selected for

this study. The technique consisted of injection of either a suspension of Indian ink or a living suspension of *mycobacterium tuberculosis*, as grown from the discharge of a person shown active disease. Intrathecal injections were given under light ether anæsthesia using a tuberculin syringe and a given size 16-gauge needle. Four groups of animals were employed.

Eleven animals died as a result of the injection. Six died two to three weeks after the injection. All these animals were examined post mortem. Both macroscopic and microscopic examination was done. Two animals showed definite hydrocephalus with dilatation of the ventricles deposition of the dye in the meninges, leucocytic reaction and in one case thrombosis of one of the venous sinuses. All the successfully injected animals showed evidence of meningeal irritation with neck retraction and skew deviation of the head. Attempts were made to visualise the ventricles in the albino rat with pyelocil, in the living, but this was a failure. Macroscopic and microscopic changes of the arachnoid granulations in various age groups have been under study.

(b) *Clinical study of 4 hydrocephalic children.*--No obvious cause was forthcoming, viz. specific infection, prenatal trauma, toxæmias of pregnancy in the mother, post natal trauma, or meningitis. Most children were quite normal at birth and showed head enlargement a little later. The obstruction was of congenital origin.

Investigation by ventriculography, indigo carmine test, C.S.F. examination with manometry, and physical, chemical and microscopic features of the fluid.

Case No.	Age	Type of hydrocephalus	C.S.F. pressure	Treatment
1	2 years	Internal	300 mm.	Ventriculo-peritoneal shunt
2	2 months	Internal	200 mm.	Died after ventricular puncture.
3	3 years	Communicating	300 mm.	Died after ventriculography meningitis.
4	2½ months	Internal	300 mm.	Ventriculo-peritoneal shunt.

Ventriculo peritoneal shunt is a simple operation and is comparatively safe in a child of poor risk. The value of ventriculo-uretric shunt in such children is also being studied.

46. Clinical and experimental studies on keloids under Prof. K.K. Ghosh, Medical College Hospital, Calcutta.

Object of work.— (1) Why and how keloids occur in certain individuals with or without operative trauma.

(2) To establish the relationship between the diffusion of extra-cellular fluids with the formation and recurrence of keloids in the selected sites.

(3) To evaluate the different modes of treatment.

In the last 6 months, 43 cases of keloids were encountered of which only 22 had primary keloids. These patients (22 only) were subjected to investigations and different modes of treatment.

On completion of detailed history taking and clinical examination, the patients were subjected to (1) ultraviolet fluoroscopic examination for determination of the nature of vascularity and diffusion of extra-cellular fluid, and (2) microscopic examination of the biopsy material before the institution of treatment for noting the histological structural detail.

The patients were then subjected to the following modes of treatment.—

(1) Deep x-ray in combination with surgery—12 cases.

(2) Cortisone infiltration in combination with surgery—7 cases.

(3) Cortisone infiltration alone—2 cases.

(4) Surgical excision only—1 case.

After the completion of treatment the patients were again subjected to ultraviolet fluoroscopic examination and the excised keloid was subjected to microscopical examination for determining the changes produced as a result of treatment.

The patients are then being followed up.

OBSERVATIONS ON THE INVESTIGATION AND TREATMENT

(1) *Ultraviolet fluoroscopic examination.*—Appearance of patchy fluorescent zones in the keloid mass and a denser fluoresence in the peri-keloidal zone in ultraviolet flurscopic examination suggests excessive diffusion of extracellular fluid at these sites. It has been observed further that the selected site where keloid usually occurs, viz., the front of the chest, the outer and anterior aspect of shoulders and a broad zone on the midline of abdomen emit brighter fluorescence than the adjoining skin surfaces in susceptible individuals.

In 9 cases there were marked diminution in fluoresence both in the keloid as well as in the peri-keloidal zone after treatment with deep x-ray. In one the fluoresence was absent altogether and in the other the fluoresence was *unaltered*.

Of the two cases treated by cortisone infiltration alone one had definite diminution whereas the other showed hardly any change.

(2) *Histological pattern and the changes produced by treatment.*—Untreated keloid has, more or less, a constant structural pattern. The skin lining the keloid shows occasional areas of parakeratosis. The skin appendages are absent in the secondary keloids but are present though in lesser number in the primary ones. The sub-epidermal zone shows the collagenous connective tissue layer where there are masses of spindle-shaped fibroblastic cells of various size and thickness, with abundant collagenous matrix. The immediate sub-epidermal zone shows loosely woven collagenous material. Blood vessels consist of endothelium lined spaces and are more numerous in the peripheral part of the fibrous tissue.

There are occasional areas of lymphocytic infiltration.

Cases treated by deep x-ray exhibited the following changes.—Minute ulceration in the epidermis. Prickle cell layer of the epidermis contained enlarged cells with granular cytoplasm. In the sub-epidermal zone there was œdema and hyalimisation of fibrous tissue which extended deeper. The layer as a whole looked more fibrous than cellular. The nuclei of connective tissue showed fragmentation. The blood vessels were lined by swollen œdematous endothelium. Sections stained by Pickworth's method showed very few vessels in the tissues.

Cases treated by cortisone infiltration.—These showed occasional brick red collagen consisting of granular amorphous cytoplasm with scattered nuclear remnants. There were occasional hæmorrhagic areas with beginning of organization.

(3) *Treatment by different methods.*—In the 1st group of the 12 cases treated by deep x-ray along with surgery none has come back with recurrence so far. All the patients had more or less relief from symptoms after the completion of pre-operative deep therapy. On an average, the cases had 1450 r in 6 days time both before and after the surgical treatment.

2nd group of the 7 cases treated by cortisone infiltration following surgical excision of the keloid, 1 has come back with complete recurrence within 2½ weeks of the completion of treatment. On an average 1½ c.c. of inj. cortilacetate (1 c.c. equivalent to 25 mg.) was infiltrated in this scar at weekly interval for 5 to 10 weeks. The others in this group are still symptom-free.

In the 3rd group, only cortisone infiltration was done at weekly interval with 1½ c.c. of inj. cortilacetate both in the keloidal as well as in the perikeloidal zone. Two patients have been subjected to this mode of treatment so far. In both there was partial relief from symptoms after the 3rd injection. In one, the regression of the paramedian abdominal scar was nearly complete after the 5th injection, whereas in the other the regression was not at all remarkable even at the end of 8th injection.

In the 4th group, only surgical excision was carried out. Only case subjected to this line of treatment came back with recurrence after 2 weeks inspite of healing by primary intention.

47. Investigation of the economic feasibility of using all-plastic, expendable blood-collecting and blood-transfusion equipment in providing a blood transfusion service for a rural area in Orissa under Dr. David R. Bassett, Dr. Balabhadra Mahapatra and Dr. Dhirendranath Mund at Distt. Sambalpur, Orissa.

Plastic expendable blood transfusion equipment has many advantages over conventional glass and rubber equipment; it is useful in all blood-banking operations, and especially in rural medical practice (e.g. Primary Health Centre), civilian and military disasters, and for specialized hematologic uses. If this equipment were available in India, it would be possible to give transfusions in rural areas, without requiring the equipment and staff time which is necessary to clean and sterilize conventional equipment. A field research cum demonstration project was set up to investigate the economic feasibility of using plastic expendable equipment in comparison with conventional equipment, plus some related aspects of blood transfusion work in rural areas.

Twenty-two transfusions were given to 15 patients between March 1956 and September 1957, using plastic expendable equipment. The average cost per transfusion was Rs. 12.55 (*plus* additional customs charges for importing equipment averaging Rs. 4.50). Some of the plastic components can be re-used for various hospital purposes, thus reducing the total cost per transfusion. An average of 78 minutes of one physician's time was required for each transfusion.

The estimated cost of conventional glass and rubber transfusion equipment prepared according to the standards established at Calcutta Blood Bank, is Rs. 8.40. It has not yet been possible to set up blood transfusion services at the Government Hospitals at Bargarh or elsewhere, so that further cost data regarding conventional equipment has not yet been obtained.

Inquiries were made on the status of manufacturing plastic medical equipment in India.

The incidence of Rh (D) negativity in the population of this area is 1.89 per cent (S.D. ± 0.24 per cent). A simple method of providing a hearted, lighted surface for Rh-testing is described.

Transfusions were given using proven group 0 blood without crossmatch. No adverse transfusion reactions occurred.

It is possible to establish a simple, portable blood transfusion service for Rs. 180. A report has been prepared describing experience at Barpali in giving transfusions under rural conditions. Eight Physicians have been trained in the practice of blood transfusion therapy.

VDRL slide test has been learned by 3 Barpali Village Service physicians, and demonstrated to 5 other physicians, and 2 laboratory technicians. The technique is simple, reliable, and can be performed with simple and inexpensive equipment costing about Rs. 165. Three hundred and eighty-three tests were performed. Thirty-five specimens

were sent to the Antigen Production Unit Laboratory for checking results ; it appears that one has a tendency to over-read tests. However, there was no case in which we failed to detect a positive reaction which was reported positive by the Antigen Production Unit Laboratory.

48. Inquiry on development of biologically specific histochemical method for the localization of pituitary hormones under Dr. V.R. Khanolkar at the Indian Cancer Research Centre, Bombay.

1. *In vitro* stimulation of corticoid synthesis under the influence of added ACTH has been studied. It is felt that coupling of oxidative phosphorylation may be necessary for optimum synthesis of corticoids.

2. A new and specific method for the histochemical localization of corticoids has been developed, which consists of oxidising the alpha-ketol grouping of corticoids with FeCl_3 to an aldehyde compound. Aldehyde compounds are then stained by leucofuchsin or Schiff's reagent.

3. Ovarian sections show evidence of FeCl_3 —Schiff positive material. This observation is likely to give valuable information regarding the pituitary-adrenal-ovary relationship.

4. A new approach for increasing the ACTH secreting cells of the pituitary is being developed. For this the animals are adrenalectomized and the adrenals transplanted in the spleen. With this procedure the renal impairment is prevented and the adrenalectomized animals appear to survive apparantly without the addition of sodium chloride in drinking water.

49. Survey of malignant lesions of the ora-pharynx in the population of Guntur District and to evaluate pre-disposing and exciting causes, if any, for the high incidence of these lesions in the area under Dr. D. Jagannadha Reddy at the Guntur Medical College, Guntur.

Outpatients of the Government General Hospital, Guntur, were examined for the presence of oropharyngeal carcinoma for the period of August to December 1957. Sixty-six cases of epitheliomata were found in the oropharynx as against 65 epitheliomata registered for the same period in sites other than oropharynx. In the latter series carcinoma cervix is found to be 66 per cent of the total. Forty-two cases of the 66 oropharyngeal carcinomata were found in the males and most of them were in the age group of 30 to 60 years. Carcinoma of the cheek and the tongue contributed to 50 per cent of the oropharyngeal carcinoma in the male, while the same formed insignificant percentage in the female. Equal incidence in both sexes but considerably less number was observed of carcinomata of the floor of the mouth, alveolar margin and the larynx. Four cases of the carcinoma palate were exclusively found in the females. One of these patients is in the habit of smoking the chutta keeping the burning end inside the mouth.

The association of smoking of chutta was significant in the majority of male patients with carcinoma cheek and tongue and they have been moderate to heavy smokers. In the female chewing of betel, with or without tobacco, was found to be of significant causal importance. In only a very minority of female patients history of smoking chutta was obtained.

In a small but negligible number, neither history of smoking nor of chewing could be elicited. History of addiction to alcohol was recorded in an occasional case or two and this bears no causal importance.

Leukoplakia was recognized in 2 cases. V. D. R. L. test was done as a routine in most of the cases and only in a small number it was positive.

Barring the isolated case of chutta, cancer observed in the female, the other three cases of palatal cancer noticed in the female are different, from that reported from Visakhapatnam.

In 25 per cent of the oropharyngeal carcinoma registered vitamin deficiency, by way of angular stomatitis, is noticed and in an equal number oral sepsis was also recognized. It is difficult to indicate whether oral sepsis preceded or is consequence of cancer.

In about a 3rd of the cases pigmentation of oropharynx was observed. This pigmentation was also noticed in the control groups in whom precancerous or cancerous lesions were not noticed but have given definite history of smoking chuttas. It is intended to carry out biopsy study of these pigmented spots with a view to recognize any pre-cancerous lesion as was suggested by Reddi and Rao (*Ind. Jour. Med. Sc.*, 11, pp. 791-798, 1957.)

During this period inpatients admitted into the City Hospital for diseases other than oropharyngeal carcinoma and also staff of the institutions were examined for oropharyngeal carcinoma. In 3,000 cases so examined no malignant lesions of oropharynx was spotted in non-smokers, or smokers belonging to the age group 30 to 70.

Verification was also made for the incidence of oropharyngeal carcinoma in various communities resident in Guntur but no significant variation in incidence of the oropharyngeal carcinoma from community to community was observed and this is probably due to common habits of smoking and chewing in various communities resident in Guntur. Carcinoma tonsils and larynx appears to be much less in number in our series when compared to those reported by Khanolkar (*Acta Un. Int. Cancer*, p.6, 881, 1950). It is proposed to carry out the survey of age group 30 to 70 in the local factories and also in certain parts of Guntur municipality with the sole object of spotting the early malignant lesions of oropharynx and record available predisposing precipitating factors.

50. Studies on the physiology of dermatophytes under Dr. A.N. Chakraborty at the School of Tropical Medicine, Calcutta.

During the period commencing from 11th May, 1957 to 20th September, 1957 the following lines of work were undertaken.—

I. *Culture of dermatophytes*.—Scrapings from the scales of the skin were taken from 20 cases of ringworm infection out of which 12 proved to be positive for *T. rubrum* culturally. Of the remaining 8 cases, 3 did not show any growth and 5 were contaminated with *Aspergillus*, *Penicillium* and *Helminthosporium*, respectively.

II. *Cytochemical studies of the culture*.—Normal cytochemical patterns of these fungi were studied. Some cytochemical entities (e.g., alkaline phosphatase, polysaccharides, calcium, iron and lipids) were studied at various ages of growth of the fungus grown on different culture media. It was seen that the reaction for alkaline phosphatase, polysaccharides and lipids were definitely more intense in the fungi grown on Sabouraud's glucose-agar media than those grown on other media (e.g., Sabouraud's maltose-agar, cornmeal-agar, nutrient agar, conservative media and agricultural media).

Localization of alkaline phosphatase was visualized by both the standard as well as by a slightly modified technique. Modified technique gave better result. Optimum time of incubation in the substrate medium was found between 3 and 6 hours.

Polysaccharides were found to be present mostly in outerwalls of the hyphae and microconidia and in inter hyphal septa.

Presence of calcium, iron and lipids has been demonstrated for the first time in *T. rubrum*. Here, too, both the techniques were employed of which the modified one proved satisfactory.

Iron, both in ferrous and ferric form, as well as in inorganic and organically bound form was observed in hyphae and microconidia.

Lipids were demonstrated by Sudan black B and Sudan III technique. Culture done on media incorporated with these two dyes showed normal growth of the fungus and concentration of the dye within the hyphae and microconidia in the form of globules.

Inorganic salts of calcium was demonstrated by standard methods both in the hyphae and microconidia as scattered grains in the cytoplasm and also in the nucleolus.

III. *Histochemical studies on the infected skin*.—Work has just been started on histochemical studies of the skin infected with ringworm and a few skin biopsy material have been collected from culturally proved cases.

HAEMATOLOGY

51. Hæmatological unit under Dr. J.B. Chatterjea at the School of Tropical Medicine, Calcutta.

I. NUTRITIONAL MACROCYTIC ANAEMIA

(A) Assay of vitamin B_{12} :

(a) *Serum vitamin B_{12} level.*—In a series of 25 uncomplicated cases, the mean values of free and total B_{12} level were 9.9 (range 0 to 82) and 96.5 (range 0 to 330) micro-microgram per ml., respectively. In 14 cases the total vitamin B_{12} level was lower than 90 micro-microgram per ml. These values are significantly lower than the corresponding values for normal Indians. After treatment with specific anti-megaloblastic drugs the level of total vitamin B_{12} definitely increased.

(b) *Vitamin B_{12} binding-capacity of serum.*—The vitamin B_{12} binding-capacity of serum was measured by estimating the serum level of free and total B_{12} following incubation *in vitro* of 1 ml. of serum with 1000 micro-microgram of B_{12} at 37°C for two hours. There was significant rise of free and combined fractions following such incubation.

(B) Folic acid assay :

(a) *In serum and urine.*—As reported last year, folic-acid levels in serum and urine vary widely and do not present any pattern significantly different from that in the control groups. It appears, therefore, that folic-acid levels in serum and urine *per se* provide no dependable guide to the existence of folic-acid deficiency.

(b) *Differential test of urinary folic-acid excretion after test dose of folic acid.*—Twenty-four hours' excretion of folic acid in urine was measured after administration in sequence of 5 mg. folic acid, (i) subcutaneously, and (ii) orally. In N.M.A., the mean excretion of folic acid after subcutaneous and oral test doses were 0.79 (range 0.19 to 1.38) and 0.45 (range 0.12 to 1.65) mg., respectively. After treatment, the mean excretion values after subcutaneous and oral test doses were 1.25 (range 0.12 to 2.3) and 0.78 (range 0.19 to 1.38) mg., respectively. With a similar technique, mean normal value after subcutaneous dose was 2.58 (range 1.45 to 3.72) mg. and after oral test dose was 1.83 (range 1.15 to 2.52) mg.

II. A THALASSAEMIC SYNDROME

During this period comprehensive investigation was done in a series of 86 cases with a view to characterize them as fully as possible. It was found that in this series there were 74 instances of Hb.E-thalassæmia, 11 instances of homozygous thalassæmia and 1 instance of Hb.S-thalassæmia.

(A) *Comparative study of Hb.E-thalassæmia and homozygous thalassæmia.*—Comparative study in these two groups did not present any differentiating clinical and hæmatological features. Homozygous thalassæmia *per se* did not appear to present any qualitative change significantly different from that seen in Hb.E-thalassæmia combination. Clear-cut differentiation of the two conditions could only be made through electrophoretic analysis of hæmoglobin.

(B) *Differential hæmoglobin pattern in Hb.E-thalassæmia disease.*—Relative proportion of the hæmoglobins E, F and A was measured in a Densitometer. Hb.F was measured by alkali-denaturation technique. In a series of 20 cases, the mean values of Hb.E, F and A were 67.1 per cent (range 45 to 83.5 per cent), 24.4 per cent (range 5.8 to 49 per cent) and 8.5 per cent (range 0 to 28.3 per cent) respectively.

These data corroborate the view that thalassæmia gene, while enhancing the expressivity of Hb.F, tends to suppress the formation of Hb.A. Expression of Hb.E is also disproportionately increased.

(C) *Family studies.*—In each of the 74 cases with Hb.E-thalassæmia disease, Hb.E in the heterozygous form was always present in either parent. Percentage of Hb.E in the heterozygous parent varied from 30 to 63.

(D) *Incidence of Hb.E and thalassæmia heterozygote in general population.*—In a series of 700 unrelated Bengalee Hindus were encountered 2 instances of Hb.EE, 25 instances of AE and 26 instances of heterozygote thalassæmia (AA).

(E) *Comparative data on Hb.E heterozygote and thalassæmia heterozygote.*—Critical analysis of various data in the two groups showed that in general, abnormalities of erythrocytic morphology were more pronounced in thalassæmia heterozygote. Owing presumably to the relatively higher percentage of Hb.E in Hb.E heterozygote (AE) over Hb.A₂ component in thalassæmia heterozygotes (AA), paper electrophoretograms in general clearly differentiated the two heterozygote conditions.

III. HAEMOPHILIA SYNDROME

In a series of 15 patients with evidence of deficient thromboplastin formation manifested by prolonged coagulation time and/or poor prothrombin consumption, the incidence of anti-hæmophilic globulin (AHG), plasma thromboplastin component (PTC), and plasma thromboplastin antecedent (PTA) deficiency was 11, 3 and 1 respectively. In addition, in one male patient there was evidence of combined deficiency of AHG and PTC.

52. Inquiry on anæmias in infants and children under Professor H.I. Jhala at the Grant Medical College, Bombay.

For the purpose of this report the analysis is divided into two parts. Part I deals with antenatal cases and their new-born. In the previous report ending September 1956, 73 cases were reported. Thirty-two cases were examined subsequently, and this work was concluded

after completing investigation of 105 cases. Part II contains the analysis of the results of investigations on anæmic infants and children. Last year 55 cases were reported. Since then 145 more children have been examined, bringing up the total to 200 cases. However, this report contains data on 150 cases as the remaining are being worked up.

Following are the total number of investigations done so far :—

Hæmatological	485	Serum Fe	206
Follow up	143	Serum B ₁₂	208
Bone marrow	148	Sickling	311
Dietary	152	Fetal Hb	178
Rh grouping	220	Paper electrophoresis	13
Serum proteins	137	Families investigated	16

53. A survey of occurrence of sickle-cell trait and sickle-cell disease or its subtypes in Mahar Community of Nagpur and its neighbouring villages under Dr. R.N. Shukla at the Medical College, Nagpur.

(A) A survey was carried out amongst the adult labour population of the local textile mills, which employ them from different districts covering roughly an area of 100 miles radius surrounding Nagpur. The labourers belong to low economic group of the local population and predominantly were from the Mahar, Kunbi, Maratha, Teli, Gond, Koshti and Mohameddan communities. In all 1,010 cases were examined. The presence of sickling was demonstrated by the method of Daland and Castle (1948). Fresh reducing solutions were made daily and the preparations were examined within two hours. All positive cases of the day were re-examined next day to eliminate false positive results. Peripheral smears were examined in all cases for morphological erythrocytic abnormalities and the presence of target cells. In cases showing the presence of target cells in peripheral smears and sickling by wet sealed preparation, hæmoglobin analysis was done by paper electrophoresis and alkalidenaturation technique for confirmation.

Results :

INCIDENCE OF SICKLE-CELL TRAIT IN CENTRAL INDIA.

Castes	Number of Persons examined	Number of persons positive for sickling	Percentage of incidence	Frequency of Sickle cell gene
Mahar	450	100	22.2	0.12
Kunbi	116	11	9.4	0.05
Teli	80	9	11.3	0.06
Gond	53	0	0.0	0.00
Koshti	46	0	0.0	0.00
Mohameddan	68	0	0.0	0.00
Brahmin	26	0	0.0	0.00
Miscellaneous	171	0	0.0	0.00
Total	1,010			

Positive sickling was present in Mahars, Kunbis and Telis. The other consisting of Gond, Koshti, Brahmin, Mohameddan and miscellaneous groups, including various other castes, did not show sickling. In Mahars the percentage of positive sickling was 22.2 and in Kunbis and Telis, 9.4 and 11.3, respectively. Paper electrophoretic studies in the positive sickling cases showed the A S pattern; thus confirming the presence of sickle cell hæmoglobin in heterozygous form.

(B) Total number of cases of sickle-cell disease detected from this area so far were seven, out of which four were of sickle-cell anæmia and three of sickle-cell thalassemia. The diagnosis of these cases were confirmed after detailed investigation, hematological (including hæmoglobin analysis by paper electrophoresis and alkali-denaturation technique) and genetical.

Experimental studies on acquired hæmolytic anæmia under Dr. N.N. Sen at the Seth S. Karnani Memorial Hospital, Calcutta.

The work done was mainly concerned with the production of antisera in rabbits against guinea pig red cells and qualitative and quantitative study of these sera produced during different periods of immunization and by different methods of immunization.

Techniques for the detection of 'incomplete' antibodies have been standardized. Rabbits have been immunized against guinea-pig red cells by varying doses, by varying intervals and by different routes of injection. Titre of the antisera by different methods and at different periods have been determined. Effect of temperature on the titre thus obtained has been investigated.

55. A study in the pathogenesis of anæmia in infections under Dr. V. S. Mangalik at the K. G. Medical College, Lucknow.

In all 75 normal healthy individuals have been investigated. These include medical graduates and students of whom 45 were males and 30 females. They varied from 65 kg. to 85 kg. in weight and in no case the hæmoglobin value was below 12.0 g. per cent and total serum proteins below 6.0 g. per cent.

1. TECHNIQUES EMPLOYED

(a) *Hæmatologic techniques.*—All estimations were carried out within four hours of the withdrawal of blood from the antecubital vein into wintrobe's double oxalate mixture. Red cells were enumerated in

Thoma's pipette in a dilution of 1 in 200, using isotonic sodium-chloride solution as the diluent. Two-pipette two-chamber method was employed as advocated by Ham and the standard error of the red cells count was determined in each case. Total leucocytes were counted in a dilution of 1 in 20, using 3 per cent acetic acid as the diluent. Hæmoglobin determinations were performed by acid hæmatin technique of Sahli. For the study of red cell morphology and differential leucocyte counts, peripheral smears were drawn on glass slides and stained with Wright's stain. Wintrobe's hæmatocrit was employed for the determination of packed red cell volume.

(b) *Biochemical techniques*: Plasma iron was determined by a modified technique of Hamilton. To 1.5 c.c. of plasma was added 0.75 c.c. of 2N hydrochloric acid and the mixture allowed to stand for 10 to 15 minutes after thorough shaking. 0.75 c.c. of trichlor-acetic acid was then added with vigorous stirring by a glass rod. This mixture was left for 20 to 30 minutes, and then centrifuged at 3,000 r.p.m. for 30 minutes. The supernatant was decanted off. To 1.8 c.c. aliquot of the supernatant were added the following in this order :

0.05 c.c. thioglycolic acid.

0.40 c.c. (0.1 g./100 c.c.) O-phenonthroline.

0.75 c.c. saturated sodium acetate.

The tube was shaken and allowed to remain at room temperature with a reagent blank (0.90 c.c. glass distilled water, 0.45 c.c. 2N HCl and 0.45 c.c. trichlor acetic acid *plus* reagents above) for 30 minutes. Optical density was measured in a Beckman's spectrophotometer model B at 510 m μ .

Total and unsaturated iron-binding capacity as also the per cent saturation of iron in the plasma calculated as suggested by Cartwright and Wintrobe (*Jour. Clin. Invest.*, 28, p. 46, 1949). For the determination of plasma copper and the copper content of the red blood cells and the whole blood, the techniques of Gubler *et al.* (*Jour. Biol. Chem.*, 196, p. 209, 1952) were followed.

Glassware used in the collection, storage and estimation for iron and copper was carefully cleaned to make it copper-iron free by washing thoroughly with soap and water, rinsing, emmersing in potent potassium dichromate—sulphuric acid cleaning solution for one hour, and then rinsing with tap-water, simple distilled water, and finally with double distilled water, prepared in a all glass still. All the reagents used, including anti-coagulants were of A-R grade and of known iron-copper content. Matching of colour was performed on a Beckman spectrophotometer model B at 510 m μ for iron and 440 m μ for copper.

Results.—The normal range of various determinations obtained on healthy individuals are shown in the Table :—

TABLE.

Determination	Number Investigated	Range	Mean Value
Red cell count/cmm. blood	55	4.1— 6.0	4.9
Hæmoglobin, g. per cent	75	12.0— 18.0	14.7
Packed red cell volume, per cent	75	38.0— 50.0	43.0
Mean corpuscular volume cubic microns	55	73 — 95	85
M. C. H. C., per cent	75	29 — 37	33
Plasma iron, microgram, per cent	75	70 —160	116
Unsaturated Iron binding capacity, microgram per cent	20	180 —260	216
Plasma copper microgram per cent	55	95 —180	145
Red cell copper „ „	15	60 —120	96
Whole blood copper „ „	15	90 —140	112

56. Inquiry on gastric secretions in pregnancy under Dr. C.R. Das Gupta at the Islamia Hospital, Calcutta.

During the short period 28th Aug. to 30th Sept. hæmatological examinations were carried out in 27 and fractional gastric analysis in 11 pregnant women attending the ante-natal clinic of Islamia Hospital. Analysis of the findings are given below :—

- Religion :* Twenty-five were Muslims, 1 Hindu and 1 Anglo-Indian.
- Age :* The age varied from 17 to 35 years; 7 were below the age of 20; 14 between the ages of 20 and 30 and 6 above 30 years.
- Parity :* Parity ranged from 1st pregnancy to 7th pregnancy; primigravidæ forming the largest number (9) of the series.
- Term :* The majority of the cases, 19 out of 27, were seen during the third trimester; 7 during the second trimester and one during the first trimester.
- Blood picture :* Examination of blood was carried out according to the technique of Napier and Dasgupta (1945).

23 of 27 pregnant women examined had some degree of anæmia; moderate degree of anæmia with red cell count below 2.5 millions per cmm. was seen in 4 cases and slight degree of anæmia with red cell count over 2.5 millions was seen in the remaining 19 cases. The type of anæmia was macrocytic in 13 cases, hypochromic in 1 and normocytic in 9.

Erythrocyte sedimentation rate according to Wintrob's method, was high in all the cases, including the cases with normal blood picture, and ranged from 30 to 68 mm. at the end of the first hour.

Gastric analysis.—Fractional gastric analysis was carried out according to the technique described by Napier and Dasgupta (1945) in 11 of the cases examined hæmatologically using 7 per cent alcohol as the test meal. Gastric acidity was found to be low in 7 and normal in the remaining 4 cases.

The number of cases studied in this very short period are too few to allow any correlated study between gastric acidity and blood picture.

CARDIO-VASCULAR DISEASES AND HYPERTENSION

57. *Inquiry on the incidence of atherosclerosis and hypertension in Delhi State and the correlation of the latter with Blood Lipids under Dr. S. Padmavati at the Lady Hardinge Medical College & Hospital, New Delhi.*

POPULATION GROUPS STUDIED :

1. *Industrial Workers.*—The investigation on the industrial workers is now complete, 500 workers from the Delhi Cloth Mills having been examined.

2. *Rural population.*—808 men and women out of a population of 23,000 from Najafgarh have been studied so far.

3. Small groups (about 25 members in each) selected on the basis of having the same occupation, belonging to the same community and, therefore, having similar dietary habits were studied. These groups were :—

1. *Low income groups.*—

(a) Hospital coolies	... 30.
(b) Gardeners	... 22.
2. *High income groups.*—

(a) Doctors (private practitioners)	... 14.
(b) Business Executives	... 20.
(c) Engineers	... 28.
(d) Sikhs	... 22.
3. *Medical students of the Lady Harding Hospital.*—

All women	... 25.
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1. *Investigations done.*—A thorough dietetic history by a trained dietitian was taken on all these cases. It was possible to correlate the results obtained by the oral questionnaire method with the weighing method of diet survey in the case of medical students and of a few families and results tallied very well.

2. A complete examination, including screening of chest and E.K.G. was done in all cases.

3. Biochemical investigations done were—

(a) Total cholesterol.

(b) Phospholipid estimations.

Results obtained.—The results obtained greatly exceeded the original objectives of the inquiry as many other data came to be recorded during the course of this survey. The results have been grouped under 4 heads for convenience.

1. *Incidence of hypertension and atherosclerosis.*—The criteria for the diagnosis of hypertension was not the arbitrary level of 140/90 mm. Hg., but was assessed on the evidence of other data on the cardio-vascular system as well (E.K.G., screening, urinalysis etc.). A fallacy that might be pointed out in assessing the incidence of hypertension in the groups is the high incidence in the high socio-economic groups due to the small number of cases in the series. This was fully realized but until the number of cases in each group is considerable, the error cannot be avoided. There was certainly a higher incidence of hypertension among the high socio-economic groups than in the lower.

It was also possible to state the normal range of blood pressure in the various socio-economic groups and to assess the effect of such factors as age and body-weight on the blood pressure. Among the low socio-economic groups who numbered 1,022 there was a very slight rise in the blood pressure with age, this trend being more marked among the females. There was, however, a much more marked rise of blood pressure both systolic and diastolic, with rise in body-weight.

Compared to figures from Western countries the blood pressures in the high socio-economic groups resemble very closely the Western figures. In the case of the low socio-economic groups the systolic blood pressures were higher in the Western candidates at every decade. In the case of the diastolic blood pressure there was not much difference up to the age of 40 after which the diastolic blood pressures were significantly higher in Western countries.

The blood pressure and body-weight were both higher in the high socio-economic group when compared to the lower.

Atherosclerosis.—The criteria used for the diagnosis of atherosclerosis was a positive electro-cardiogram indicative of coronary artery disease. In the absence of post-mortem data, this was the only possible criterion. The same arguments about the small size of groups used in the case of hypertension apply to atherosclerosis. The incidence was much higher among doctors and business executives than the other groups.

2. *Dietary habits.*—Using the questionnaire method, the diet of 203 individuals of high and low socio-economic groups was worked out. This diet survey was compared to a similar survey made in 1938 among rural groups in Delhi and in 1946 in the Lady Hardinge Medical Students' Hostel.

Among the high socio-economic groups, the diet was adequate in calories, sometimes in excess of the energy requirements. Both among vegetarians and non-vegetarians the diet was deficient in animal protein and the fat content tended to be rather high in 2 out of 4 members of this group. The percentage of calories derived from fats was highest among the medical students followed by the engineers, doctors, Sikhs and businessmen in that order. The body-weight was also higher in these groups than in the low socio-economic groups. The percentage of calories derived from fats averaged from 30 to 40 per cent.

In the urban low socio-economic group, the diet was adequate in calories but very deficient in animal protein and low in fat.

Among the rural low socio-economic groups, the men had a diet that was adequate in calories and protein but deficient in many items such as vegetables, fruits, eggs, meat and fish. The percentage of calories from fats was 27.5. The rural women had an inadequate diet in calories, protein and fats. Compared to the previous dietary surveys mentioned, the rural diet had improved, whereas that of the medical students had not.

3. *Blood cholesterol levels.*—The average blood cholesterol levels were considerably lower in the low socio-economic groups than in the higher. The factors that might be responsible for this higher blood cholesterol level may be :—

- (a) Greater body-weight in the high socio-economic groups.
- (b) Higher fat intake in these groups.
- (c) The higher age in these groups.

Until a larger number of individuals are studied in each group, it would not be possible to work out the relative effect of these 3 factors.

58. Study of environmental and nutritional factors affecting the incidence of atherosclerosis and coronary heart disease in Indians under Dr. K.S. Mathur and Professor P.N. Wahi at the Medical College, Agra.

During the year under review, 553 cases of coronary heart disease treated by one of us (K.S.M.) have been analysed. The constitutional, environmental and nutritional factors as far as available, have been worked out. This has helped us in our present work where we go into more details concerning the role played by different factors in the pathogenesis of coronary heart disease.

For the purpose of present study, we are investigating two groups of cases.

Group I.—Established cases of coronary heart disease, including cases of myocardial infarction, angina pectoris and coronary failure which

we study on *proforma* I in great detail and do the pathological biochemical, fluoroscopic and electrocardiographic investigations. While studying the hereditary factors we are now doing the blood grouping of these patients as well. We have studied 60 cases of coronary heart disease. Included in group I are also cases of hypertension because of its close association with coronary heart disease. These cases are also studied on *proforma* I. We have studied 66 cases from this group. Complete analysis of diets with regard to caloric intake, percentage of calories derived from fat, the varieties of fats ingested and amount of cholesterol are done in all cases.

Group II.—Includes at random study of general population. For this purpose all the persons examined are categorized into five subgroups (a) Professionals, executives and big businessmen, (b) clerical workers, (c) industrial workers, (d) manual workers and (e) agricultural workers. These are studied on *proforma* II. Complete family history, personal history, dietary history is elicited in all the cases and clinical examination with special reference to heart and blood pressure is made in them. It was not possible to do electrocardiography of all the cases in the beginning but now electrocardiograms are taken as a routine. The diet is analysed on the same basis as for group I and blood-lipid studies are done in as many cases as would allow blood examination. So far 416 persons have been examined which includes 146 professionals, 78 clerical workers, 146 industrial workers, 24 manual workers, and 22 agricultural workers.

It is now our aim to examine 500 persons from each of these subgroups before drawing any conclusions.

59. Study of the pattern of coronary circulation by infection technique with special reference to its relationship with the incidence of coronary heart disease under Professor P.N. Wahi and Dr. K.S. Mathur at the Medical College, Agra.

The hearts obtained were from all age groups. Five hearts between the age group 11 and 20, 6 hearts between the age group 21 and 30, 8 hearts between the age group 31 and 40, 5 hearts between the age group 41 and 50, and 7 hearts between the age group 51 and 60 years. The hearts obtained were from medico-legal as well as routine pathological post mortems. In most of these cases the cause of death was trauma. The remaining hospital cases had a clinical diagnosis other than coronary heart disease.

Out of these 31 hearts, 21 belonged to type I (right coronary predominating), 5 to type II (balanced hearts with right and left supplying almost equal areas) and 5 to type III (left coronary predominating).

A gross study of these 31 hearts revealed atheromatous plaques in 8 cases.

Seven hearts showed the evidence of occlusion of the coronary artery. Out of these, in 3 left coronary and in 3 right coronary artery was involved. The remaining one had both the left and right coronaries occluded. Gross infarct was not seen in any of these hearts.

In 3 cases there was an abnormal opening in the aorta near the opening of the right coronary artery indicating an abnormal opening of the first ventricular branch of the right coronary artery.

60. Inquiry on the diagnosis of pre-symptomatic diabetes and hypertension under Dr. K.S. Sanjivi at the Madras Medical College, Madras,

Hypertension and diabetes are common familial diseases in South India. Since, by the time the subject presents himself with symptoms of either disease, the disease itself is advanced, it is desirable to have a method of detecting the proclivity to development of either condition in apparently healthy people with a family history of either disease.

The purpose of the study has been to find out if cortisone produced a temporary diabetic or hypertensive state (by its recognized physiological effects) in a significantly larger number of those with a family history of diabetes or hypertension than in those without such a history.

Twenty male volunteers of age ranging between 18 and 28 years were studied in each of 3 groups :

1. With a family history of diabetes.
2. With a family history of hypertension.
3. Without a family history of either disease, acting as controls.

The subjects, who were all on their usual diet, were given 100 mg. of cortisone daily for 7 days, after a preliminary period of observation. Significant alterations in weight, B.P., and blood sugar were observed from day to day in all the groups but not between groups. After eliminating the physiological effects of cortisone there was, however, a significant difference between the groups. This observation suggests that a study of the physiological measurements in a large series of descendents of hypertensives and diabetics as compared with normals may prove interesting and useful.

During the period of cortisone administration, a significantly larger percentage (25 per cent) of the 'diabetic' group exhibited post-prandial glycosuria, for a larger number of days than the other two groups (10 per cent) put together. Taking into account the results of the post-lunch examination of urine and the number of days on which reduction was obtained, comparison between the diabetic group and the non-diabetics shows that there is a significantly greater liability for diabetics to have post-prandial glycosuria after cortisone administration.

It is predicted that the 25 per cent of the diabetic group who exhibited glycosuria are more likely than the remaining 75 per cent to develop frank diabetes, ultimately. If a long-term follow-up confirms this, cortisone may serve as a tool in unravelling the tendency to future diabetes.

In the doses in which it was given, cortisone did not unravel any tendency to hypertension in the volunteer-group with a family history of high blood pressure.

61. Studies to investigate the incidence and possible etiological factors of rheumatic heart disease in Simla hills under Dr. Devi Chand at the Himachal Pradesh Hospital, Simla.

It is universally accepted that the basic lesion produced by rheumatic process is a protracted proliferative carditis. The mechanism of the production of this fundamental manifestation is not at all clear, in spite of the voluminous work done, particularly since the Second World War. The balance of evidence incriminates beta *Streptococcus hæmolyticus* in the production of the exudative manifestations of the disease, On account of its close association, high immune responses to it, and the experience that it is possible to prevent these manifestations by anti-streptococcal measures, this organism has been held as the possible causative agent of the disease by a large majority of workers. But all these workers are also agreed that the knowledge of the mechanism of the 'non-suppurative phenomena' is completely lacking. These include the progressive carditis which is the fundamental lesion, and until and unless a particular concept clearly explains the whole truth of a particular disease situation, it cannot be assigned the status of the direct 'cause and effect' relationship which a comprehensive concept of etiology should achieve. In fact, there are some solid facts about the rheumatic syndrome which require more thought and precision, e.g. the marked hereditary proclivity, profound effects of environment and precipitation of the syndrome in the bacteriologically negative cases by trigger mechanisms other than streptococcal infection.

The disease in all its phases is seen all over our country. Its incidence as reported by various workers has been reviewed by the author (*Ind. Heart Jour.*, Jany., 1956). During his work in the hills surrounding Simla, it was found, that the incidence of this disease was particularly high. The present investigation has been undertaken to determine the possible epidemiological factors responsible for this increased prevalence in those areas. The opportunity has also been utilized to study the clinical facets of the disease and to determine the precipitating triggers, as far as possible, and correlate them with bacteriological and immune body findings.

MATERIAL AND METHOD.

Patients suffering from rheumatic fever and rheumatic heart disease who attended the provincial hospital of Himachal Pradesh at Simla between 1954 and 1957 have been studied in detail, according to a specific *pro forma* designed for the purpose.

The incidence has been assessed on hospital cases on the basis of the total patient populations extricating medical cases and from them the cardiological cases, out of which the percentage of rheumatic cases has been worked up. On this basis the rheumatic cases are found to constitute just above 50 per cent of the total cardiological cases in the hospital population.

During the above period 200 cases have been subjected to exhaustive study on the lines explained above. These include 9 cases in the pre-rheumatic phase, 31 cases in the classical phase, 155 cases in the

phase of protracted carditis and 5 cases in the quiescent phase. Studies reveal that only about 45 per cent of the cases suffer from the acute classical attack and on age grouping the prevailing impression that majority of these attacks occur in the age groups of 6 to 20 years is confirmed. In the rest of the cases, i.e. 55 per cent the syndrome starts with low grade symptoms. Studies indicate that a pre-rheumatic phase can occur in children below 5 years. Slight predominance of the females is made out. A heavy hereditary proclivity (53.2 per cent positive) is brought out by the study and out of the geographical factors in hilly areas a relationship to cold climate is definitely discernible. In micro-climate, the factor of housing structure does not appear to be statistically significant but overcrowding seems to play a part. A number of cases, roughly one out of 3 cases studied, has shown some type of an emotional upset in the background. This appears to be an important factor in the environment, as revealed by this study. Triggers have been worked up with special attention in cases seen in classical phase, and also in the re-activated cases during the phase of protracted carditis. Careful histories reveal some definite triggers other than streptococcal infections, responsible for exciting the classical attack and re-activation in some cases. Out of the 28 investigated cases of classical attack, as many as 12 cases showed such trigger mechanisms clinically. Some of them were persistently negative on throat cultures, but in the few where antistreptolysin titres have been worked up so far, all show considerably elevated titres. More supplies of streptolysin O are being obtained from abroad as also type specific sera and it is proposed, to continue these studies further at the bacteriological department of the Lady Hardinge Medical College, New Delhi.

62. Inquiry into artificial hæmopericarditis as a means of revascularising the myocardium under Dr. G.S. Karai at the S.S.K.M. Hospital, Calcutta.

An inquiry was instituted as a long term experiment using monkeys as the experimental animals. The aim was to produce an aseptic foreign-body reaction pericarditis using a standard chemical substance. Sterile magnesium trisilicate powder 75 mg. to 100 mg. was instilled into the pericardial sac either as powder or as suspension in saline, in 3 animals, one animal being kept as control with N. saline only. The standard reaction produced was to be compared histologically as regards vascularity and granular reaction produced with other substances to be used for comparison.

The pericardium was reopened at 6 weeks to 3 months interval in different cases, and biopsy was obtained of both pericardium and myocardium. Four animals had whole blood instilled—2 c.c. of autogenous blood in 2 cases and 2 c.c. human blood in 2 cases.

Finally, 3 cases had powdered asbestos instilled as suspension of 75 mg. to 100 mg. in 2 c.c. saline and similarly biopsied for histology.

RESULTS

(1) Trisilicate and asbestos both produced a diffuse granulation and foreign-body reaction where vascularity per field was increased in

the vicinity of microscopic crystalline or fibre substance. Inflammatory cells, macrophages and foreign body giant cells and new formation of blood vessels were seen. This did not indicate, however, the direction of blood flow in these vessels.

(2) Whole blood whether autogenous or heterogenous did not produce adhesive pericarditis beyond a thin layer of fibrin, which was evidently not vascularized.

63. Inquiry on the role of adrenal cortex, stress and cholesterol in atherosclerosis under Dr. S. Sachdev at the M.G.M. Medical College, Indore.

Fifteen Indian birds (cockerels), fifteen weeks old were procured and were divided into three groups as under :—

- (i) Normal group on synthetic diet.
- (ii) Animals on synthetic diet containing 0.25 per cent of cholesterol.
- (iii) Animals on synthetic diet receiving 0.25 per cent of cholesterol and subjected to stress :—
 - (a) Injections of formalin in two birds.
 - (b) Injections of adrenaline in other three birds.

The synthetic diet as recommended by Department of Agriculture, Bombay State, for feeding chickens from two months to commencement of laying period was administered to these birds.

A complete weight record of these birds was kept and serum-cholesterol estimations were done on these animals at the time of their sacrifice.

The values of serum cholesterol in group (i) ranged from 118 mg. to 166 mg. per cent with an average of 150 mg. per cent. In group (ii) the values ranged from 170 mg. to 201 mg. per cent with the average value 187.8 mg. per cent. In group (iii) animals subjected to stress (formalin) serum-cholesterol values ranged from 232 mg. to 240 mg. per cent with the mean value 236 mg. per cent, while in animals given adrenaline the values ranged from 250 mg. to 290 mg. per cent with the mean value of 274.6 mg. per cent.

On analysis of the above groups, it would be observed that a rise of 70 per cent in the serum cholesterol was obtained in group (iii) during a period of above 40 days. The birds in group (ii), on the other hand, showed only 24 per cent increase on an average.

The heart along with the main blood-vessels and abdominal aorta were removed after sacrificing the animals, and they were studied for evidence of atherosclerosis. Macroscopic examination of the heart and the blood vessels revealed atherosclerotic lesion in 80 per cent of the animals in group (iii). Lesions were mainly in the forms of fatty streaks and in two birds atheromatous ulcers were observed.

These findings were corroborated by the microscopic studies. The lesions were observed either as localized deposits of fat droplets under

the intima extending for a short distance into the media or as typical atheromatous ulcers.

From the above observations it can be postulated that stress plays an important part in cholesterol metabolism and atherosclerosis.

64. Inquiry on pulmonary hypertension under Dr. K.K. Datey at the Seth G. S. Medical College, Bombay.

Sixteen cases of pulmonary hypertension of varied etiology, have been studied. In each case, the diagnosis of pulmonary hypertension with its etiology was arrived at clinically. Each case was fully investigated including an electrocardiogram, a teloradiogram and right heart catheterization was carried out.

The causes of pulmonary hypertension can be broadly divided into two groups --pre-capillary and post-capillary. In our series, there were 9 cases in the former group and 5 in the latter group. In post-capillary group, the causes of pulmonary hypertension, were aortic regurgitation (one case), mitral stenosis (3 cases) and constrictive pericarditis (one case). In the pre-capillary group, there was one case of V.S.D., 4 cases of A.S.D., 2 cases of P.D.A. and one each of cor-pulmonale and primary pulmonary hypertension. There was pulmonary hypertension in 2 cases of Lutembacher's syndrome—high P. A. pressure is evidently due to both pre-and post-capillary factors.

Out of four cases of atrial septal defects, two had marked increase in pulmonary resistance (680 and 1800 dynes-sec-cm⁻⁵). Both these cases showed reversal of shunt, one at rest and other one on exertion. The increase in pulmonary resistance reduced the left to right shunt as expected. In one case of A.S.D. the pulmonary resistance was almost normal (144 dynes-sec-cm⁻⁵) with left to right shunt of about 18 litres/minute. Here, the pulmonary hypertension was purely due to increased pulmonary flow.

There was one case of ventricular septal defect. This patient also had dextroversion of heart and persistent left superior vena cava. He had high pulmonary resistance (1440 dynes-sec-cm⁻⁵). This could be of congenital origin or the result of increased pulmonary flow. Because of the increase in pulmonary resistance, his left to right shunt must be minimal. This was, in fact the case. The patient actually had bi-directional shunt at the ventricular level.

There were two cases of patent ductus arteriosus. In one left to right shunt was reduced to 0.5 L/min. because of marked increase in pulmonary resistance (1040 dynes-sec-cm⁻⁵), while in the other case, aorto-pulmonary shunt was about 8.5 L/min. With this much shunt, even at age of 35 years, pulmonary resistance was normal (72 dynes-sec-cm⁻⁵) and pulmonary artery pressure showed only the upper limits of normality (This was confirmed at operation).

In the above cases, whether the pulmonary hypertension is due to increased pulmonary flow or due to persistence of foetal resistance, is a moot point. There were two cases, in which pulmonary resistance was

not increased, suggesting that resistance present during the neonatal period, does not always persist.

In one case, the pulmonary resistance was increased to 960 dynes-sec-cm⁻⁵, but no cause for it could be detected and it appears to be a case of primary pulmonary hypertension. In one case of cor-pulmonale, the pulmonary resistance was increased to 800 dynes-sec-cm⁻⁵.

All the three cases of mitral stenosis had increased pulmonary resistance—377, 1560 and 416 dynes-sec-cm⁻⁵. Pulmonary hypertension is probably mainly on the basis of increased left atrial pressure. All these patients have been subjected to surgery and the pulmonary resistance will be determined again, about 6 months later. In another case, the diagnosis was aortic regurgitation. Here, the rise in pulmonary pressure is evidently on the basis of left ventricular failure. In constrictive pericarditis, it is due to defective filling of left ventricle with back pressure in the pulmonary circuit.

There were two cases of Lutembacher syndrome. In such cases, pulmonary hypertension is due to two factors : (i) back pressure due to mitral stenosis, and (iii) increased pulmonary flow through atrial septal defect.

In general, it could be stated that increase in pulmonary resistance roughly paralleled the rise in pulmonary artery pressure, in cases of post-capillary hypertension. In cases of left to right shunt, there was greater rise in P.A. pressure as compared to that of pulmonary resistance, probably because of the additional factor of increased pulmonary flow.

Clinical findings.—The usual clinical findings, due to pulmonary hypertension *per se*, in this series were, prominent 'a' wave in the jugular venous pulse unless complicated by tricuspid incompetence, right ventricular pulsations and right ventricular heave, systolic ejection click and systolic ejection murmur in pulmonary area with a loud pulmonary second sound.

Systolic ejection click was heard in majority of the cases. Right ventricular heave was seen in all cases, except in cor-pulmonale, constrictive pericarditis and one case of atrial septal defect. In former two, emphysematous lung and pericardial constriction masked the right ventricular heave, respectively. In the case with A.S.D., though there was no heave, right ventricular pulsations were felt. This was due to increased pulmonary flow (21 L/min.) without significant rise in P. A. pressure. It was also noted that the intensity of the heave increased with rise in pulmonary artery pressure. Pulmonary second sound was loud in all cases except two : (i) cor-pulmonale and (ii) primary pulmonary hypertension. In the former it may be due to emphysematous lung masking the intensity of the sound; in the latter, it was probably due to pulmonary incompetence. In this case P.A. pressure was 110/52 mm. of Hg. In one case of mitral stenosis, pulmonary incompetence was noted at pressure of 50/30 mm. of Hg.

Correlation of electrocardiographic findings with pulmonary artery pressure.—An attempt was made to correlate the hemodynamic data, with the electrocardiographic pattern. It must be pointed out that the

number of cases in this series, is as yet, very small, and hence only general remarks, regarding the correlation can be made.

From E.C.G. point of view, attention was given to the following:—

1. Q-R-S. patterns in chest leads.
2. Height of R wave in V_4R and V_1 .
3. S-T. and T wave changes in precordial leads.
4. Height of P wave in lead 2 and 3.
5. Transitional zone.

The cases were divided into congenital and acquired group with or without other complicating lesions. Electrical forces of left ventricle, neutralized and blocked the appearance of right ventricular potentials, e.g. in the case of aortic regurgitation with left ventricular hypertrophy, there were no signs of right ventricular hypertrophy on the electrocardiogram, even though the pulmonary artery pressure was 56/45 mm. of Hg. The systemic pressure was 190/20 mm. of Hg. Similarly, in the case of ventricular septal defect, R/S ratio was less than one, in V_1 , inspite of pulmonary pressure being 80/42 mm. of Hg. Only in V_4R , R/S ratio was greater than one suggesting some evidence of R.V. hypertrophy.

With systolic overload of the right ventricle, right ventricular hypertrophy pattern appeared. In this series, R.V. hypertrophy was seen even at P.A. pressure of 45 mm. and in another case at ventricular mean pressure of 20 mm. Hg. Complete or incomplete RBBB pattern was seen with the diastolic load of the ventricle, due to increased flow, through the ventricle. This pattern was seen in all cases of A.S.D. and Lutembacher's syndromes, in this series. However, one case of mitral stenosis also showed rSR pattern in V_4R and V_1 , at a pressure of 50/30 mm. Hg. Its genesis remains to be determined. In 9 cases, out of 16, QR pattern was seen in V_4R and V_1 . It is believed that this pattern might be due to marked clockwise rotation of heart. However, all the cases did not show such marked clockwise rotation. Thus, its genesis is still unknown.

In cases of mitral stenosis, it was found that the height of R waves in V_4R and V_1 rose with rise in mean ventricular pressure. In one case, the pressure was higher, as compared to the height of R wave, probably because of associated 'myocardial factor.' ST depression and T wave inversion were seen in V_1 , V_2 and in leads to the left, as the ventricular pressure rose. In mitral stenosis the height of R in V_4R and V_1 is more or less proportional to the R.V. pressure while in A.S.D. it may not be so.

Height of P wave in leads II and III.—It was 3 mm. or more in cases of A.S.D. and Lutembacher's syndromes. In rest of cases, it was between 2 and 2.5 mm., except in the case of constrictive pericarditis.

65. Inquiry on cardiac metabolism under hypothermia under Dr. Sita Ram Kapoor at the K.G. Medical College, Lucknow.

Mongrel dogs were cooled by immersion in ice-water under intravenous chloralose anaesthesia. Dogs were hyperventilated with air throughout the experiment.

Dogs were re-warmed safely from a temperature of 24°C. At this temperature heart tolerated venous occlusion up to 13 minutes and even right auricle exploration did not produce ventricular fibrillation during this period of venous occlusion. When venous occlusion was, however, followed by aortic occlusion, ventricular fibrillation occurred as early as 1 to 7 minutes.

Prostigmine (1/4000 sol.) injected in the root of the aorta proximal to the clamp in the dosage of 0.05 c.c./kg. body-weight did not check ventricular fibrillation in any of the experiments. 2 c.c. of potassium chloride (Em. Eq/L) injected as above did stop ventricular fibrillation in all the experiments, and in two out of the six experiments did so far 0.2 c.c. of two per cent calcium chloride, injected in the root of the aorta did restore the normal rhythm.

66. "Electro-retinography in cataract glaucoma and detachment of retina" under Dr. R.P. Dhanda at the M.G.M. Medical College, Indore.

1. To study the variations in Electro-retinogram in different stages of Cataract formation and after different methods of Cataract Extraction and thus to assess their prognostic value.

2. Similarly to study various types of Electro-retinogram in different types of Glaucoma as well as in cases of Detachment of Retina again and to assess their prognostic value.

ELECTRO-RETINOGRAPHY IN CATARACT

In this series 56 eyes with different grades of Lens opacity were investigated by electro-retinography and the following table gives a summary of the observations in comparison with the findings in normal Indians of similar age group, reported earlier :

	Normals above 40 year's age	Immature cataract	Mature cataract
No. of eyes	24	26	30
Average b-pot.	0.290 mV.	0.262 mV.	0.260 mV.

The average b-potential of 30 eyes with mature cataract was 0.260 mV. and in 26 eyes with immature cataract, the average b-potential was 0.262 mV.

This suggests that b-potential may be reduced by the formation of cataract but more important to note is that a good amount of electric potential can be recorded in cases of mature cataract where the vision is reduced to hardly hand movements. This implies that even a very

small amount of light passing through a nearly opaque lens and the spongy Iris is enough to bring the Retina to appreciable activity.

Post operative changes in electro-retinogram indicate still more interesting observations. Electro-retinograms have been repeated 15 days after the operation in 18 cases. Of these, 10 after Intracapsular operation, 7 after Extracapsular operation and 1 after Currette evacuations

In all the 10 Intracapsular cases the average b-potential after operation increased in every case. In Extracapsular cases the b-potential was decreased after operation in six and unchanged in one. The next table gives a summary of the observations :—

No. of eyes	Type of operation	Av. b-pot. before operation	Av. b-pot after operation	Average variation
10	Intracapsular	0.273 mV.	0.309 mV.	+0.036 mV.
7	Extracapsular	0.254 mV.	0.228 mV.	—0.026 mV.
1	Currette evac.	0.290 mV.	0.240 mV.	+0.050 mV.

This observation possibly leads one to suggest that the retinal activity is disturbed more in cases of Extra-capsular operation than in Intra-capsular. This may incidentally explain that the breaking of suspensory ligament in Intra-capsular method does not pull on the Retina and therefore does not effect its function. It may even be hypothesised that the so called increased frequency of detachment of Retina after intra-capsular surgery may be incidental rather than factual.

Electro-retinography could help in detecting senile macular disease so commonly associated with cataract and equally commonly unpredictable because neither a peep into the fundus is possible nor the pupillary reaction a dependable guide. It would be apparent that the scotopic electro-retinogram representing the rod function as practised by Karpe and at this ERG unit will not be adequately indicative of the extent of macular diseases which is predominantly a cone pathology rather than rod one.

Flicker sensation and colour perception being the special functions of the macular area, Flicker electro-retinography and spectro stimulus electro-retinography have been studied on patients of macular disease with more dependable results. Red stimulus photopic electro-retinogram and flicker electro-retinography are therefore suggested as possible tests for macular function in cases of cataract.

II. ELECTRO-RETINOGRAPHY IN GLAUCOMA

Glaucoma is another condition where electro-retinography may help the explanation of those aspects of pathology, which it has still not been

possible to understand and may also contribute towards the assessment of prognosis in certain circumstances. Studies of Glaucomatous eyes at this ERG unit comprised 25 cases, 16 with chronic Glaucoma, 6 with secondary Glaucoma and 3 of absolute Glaucoma. The next table gives the electrical response in relation to the degree of Tension and the type of Glaucoma.

No. of eyes	Type of Glaucoma	Average intra-ocular tension	Average b-pot.
16	Chronic Glaucoma	52 mm. Hg.	0.202 mV.
3	Absolute Glaucoma	75 mm. Hg.	0.120 mV.
6	Sec. Congestive Glaucoma	90 mm. Hg.	0.275 mV.

The presence of fare amount of b-potential even in cases of absolute Glaucoma with no perception of light is strongly suggestive that the loss of vision in Glaucoma may be mainly due to the effect of raised tension directly on the optic nerve rather than on the retina. It appears that retina can stand the effect of prolonged and marked rise of tension and the bi-polar cells of the retina, from which the positive part of electro-retinogram is presumed to arise, can continue to produce the electric potential.

III. 5 patients each with *detachment of Retina* in one eye, the other eye being normal were investigated. The electro-retinogram in most of the diseased eyes was either extinguished or showed very low electrical response (Less than 10 mV.). Obviously the prognosis in these was very poor and so surgery was not indicated. Detachment of Retina is an uncommon condition in this country and most of the cases are seen in a late stage. It would be interesting to investigate the case in early stage when prognosis is good and study the change in electro-retinogram after operation.

67. Liver diseases research unit under Dr. P.N. Wahi at the S.N. Medical College, Agra.

I. *Adrenal cortical function in human liver disease.*—In a study of the role of the adrenal cortex in clinical liver disease, started last year adrenal cortical function was assessed in 22 cases of portal cirrhosis and 26 cases of infectious hepatitis. These cases were in addition to those investigated last year. Adrenal function was assessed by a battery of tests as follows :

1. Eosinophil response to ACTH administration.
2. Urinary uric acid response to ACTH administration.

3. Serum cholesterol response to ACTH.
4. Serum-sodium level.
5. Urinary 17-hydroxy-corticosteroids.
6. Urinary 17-ketosteroids.

All these tests have been shown to be reliable indices of adrenal cortical function in recent reports in the literature. From the data obtained in this study so far, it is apparent that several of the cases of cirrhosis and hepatitis are suffering from a state of adrenal insufficiency, probably confined to the 'gluco-corticoid' fraction of the hormones.

II. *Study of pattern of amino acid excretion in liver disease.*—Paper partition chromatographic studies of urinary amino acid excretion pattern were carried out in 22 cases of portal cirrhosis and 26 cases of infectious hepatitis. These cases were in addition to those reported in our last year's report. The object of these studies is to determine if a pattern characteristic of these two diseases occurs. Both the qualitative detection of these amino acids by the double-dimensional technique as well as their quantitative estimation by the circular-chromatographic method were carried out. A characteristic increase in the urinary excretion of certain amino acids was repeatedly observed in some of these cases.

III. *Study of the role of adrenal cortex in histogenesis of dietary cirrhosis in rats.*—The role of adrenal cortex in cirrhosis induced in albino rats by feeding a high-fat low-protein diet was studied in the different stages of evolution of the disease. Adrenal cortical function was assessed by studying the depletability of the adrenal ascorbic acid at each stage of the disease. It was observed that adrenal function was normal in the stages of fatty infiltration and early fibrosis of the liver, but in the later stages of the cirrhotic process it was deranged.

IV. *Studies on the clinical, histopathological, biochemical and hormonal aspects of kwashiorkor.*—Seventeen cases of kwashiorkor were investigated during the year in a combined study of the clinical, histopathological, biochemical and hormonal aspects of the disease. The clinical study revealed the presence of the typical symptoms and signs of the disease. Liver biopsy was performed in 12 of the 17 cases, the commonest pathological histology being the presence of mild degree of fatty infiltration in the periportal zones and absence of fibrosis. A battery of liver function tests was employed to assess the functional liver damage. Paper partition chromatographic studies of the urinary amino-acid pattern was done in 12 out of 17 cases. It is of interest that in only one case there was presence of amino aciduria, while in all other cases amino acid excretion was decreased. Adrenal cortical function studies showed the presence of adrenal insufficiency in some cases and normal adrenal function in others.

V. *Registry of hepatic pathology.*—The Registry has added 151 new cases during the year under review. The total number of cases registered till date are 603. All these cases are accompanied by descriptive protocols and have been indexed. The Registry also exchanged during

the year material with American Registry of Hepatic Pathology and other foreign workers.

VI. *Bibliographic index* —The bibliographic index of articles on liver diseases in current journals is being maintained and cyclostyled copies of the index up to June, 1956 will be circulated very shortly.

68. Inquiry into the changes clinical and pathological that are present months or years after apparent recovery from nutritional dystrophy or kwashiorkor under Dr. S.T. Achar at the Institute of Pediatrics, Government General Hospital, Madras.

Twenty-two children who had kwashiorkor some years previously and who were examined in previous years from time to time were re-examined this year. Of these, one was a boy of 7 who showed marked hepatic fibrosis last year. None of this group showed clinical evidence of cirrhosis of the liver or histologic evidence of increase in fibrous tissue formation.

There has been one new case this year of pronounced hepatic fibrosis of the nutritional type (without previous history of kwashiorkor) similar to the ones encountered and reported in previous reports.

There is a high incidence of persistent hepatitis and cirrhosis following what looks like viral hepatitis in children in Madras—but these children are not from the poorest economic group from whom kwashiorkor cases come.

69. Studies on anti-fatty liver fractions of the pancreas and their scope in the prevention and treatment of fatty livers under Dr. J.C. Sachdev at the M.G.M. Medical College, Indore.

Anti-fatty liver fractions and lipociac were tested for their solubility in water, ether, alcohol, hydro-alcoholic solutions, dilute acids and saline solutions. They were found to be soluble in water, alcohol, hydro-alcoholic solutions, dilute acids and alkalies but not ether.

They were found to be protein in nature and gave positive, biuret, xanthoproteic, mercuric nitrate and sulphur reactions.

Preliminary proteolytic activity studies with anti-fatty liver fractions using alkaline casein and calcified-milk method gave positive results. This was confirmed by gelatine formol method. This proteolytic activity was almost similar to trypsin. Lipocaic was found to be inactive in this respect.

On chromatographic analysis studies, it was observed that fraction A moved along the paper and gave a spot with Rf value 0.93, having proteolytic activity on x-ray film which corresponded to the spot observed for trypsin, indicating thereby that the fraction A contains a

constituent corresponding to trypsin. Lipocaic did not give any spot having proteolytic activity indicating that either it does not contain any proteolytic enzyme or it is so weak that it does not give a spot on x-ray film.

Amino-acid pattern studies of a anti-fatty liver fraction, lipocaic and trypsin were undertaken using paper chromatography. It gave distinct spots for aspartic acid, glycine, histidine, valine, and leucine. Another spot not corresponding to any known amino acids, but probably due to some peptide, could be observed. Chromatograms with lipocaic, anti-fatty liver fraction and trypsin were exactly similar and could be superimposed indicating these are similar or contain similar substances.

It could be presumed that probably these fractions and trypsin are allied substances or the fractions are mainly composed of a substance similar to trypsin. Corroborating this with the results of previous studies it can be presumed that these fractions and lipocaic contain a proteolytic enzyme similar to trypsin in active or inactive form and have a similar amino-acid composition

70 Inquiry on the development of ascites and its relation to portal vein pressure etc., under Dr. R. M. L. Mehrotra at the Medical College, Lucknow.

The Liver Diseases Sub-Committee at its Mysore meeting suggested that the work on the research scheme should be mainly directed towards a study of factors concerned in the pathogenesis of ascites and that this work should be combined with a study on balloon-cell appearance of liver cells as found in certain conditions. The work done is, therefore, presented in two parts.

PART I.

Pathogenesis of ascites in CCL₄ cirrhosis.—During the current year of this inquiry the effect of sodium retention and antidiuretic hormone on the development of ascites in CCL₄ cirrhosis was studied.

Sodium retention.—Series of animals were fed on a high sodium diet and were also given injections of DOCA (0.15 mg. per rat) on every alternate days. Cirrhosis was produced by repeated injections of CCL₄ in these animals as in the other groups. Our findings show that retention of sodium appears to influence the volume of fluid which accumulates in the peritoneal cavity of these animals. Such large accumulations of fluid as found in DOCA-treated animals were not observed in the untreated group. Our observations suggest that the sodium-retaining hormone of the adrenal cortex appears to operate at a stage when the liver is grossly damaged and when fibrosis is marked. But animals with less severe fibrosis did not develop ascites even after DOCA administration and not all animals given DOCA and showing severe cirrhosis develop ascites. These latter findings thus, suggest that sodium retention is perhaps not primarily concerned in initiating the process of fluid transudation but appears to play an important role in

increasing the volume of the transudate when the latter starts appearing in cases of cirrhosis.

Anti-diuretic hormone.—In this group cirrhosis was produced in albino rats by repeated injections of CCL_4 and antidiuretic hormone of the pituitary was administered daily during the last 5 weeks of the experiment. Our findings show that in animals treated with ADH, the incidence of ascites was not significantly higher than in the control group.

PART II.

Nature of balloon cell appearance of liver cells.—The balloon-cell appearance of liver cells as produced by prolonged administration of para-amino-salicylic acid was studied. Albino rats were used and fed a diet containing 5 per cent of sodium PAS. Our experiments showed that balloon liver cells appear in the peripheral and the midzones of the hepatic lobules within a month of PAS administration. This change becomes diffuse and involves all the liver cells of the lobule by 4 months. If the administration of PAS is, however, continued the balloon appearance of cells tends to disappear.

The balloon cells show an empty cytoplasm with almost complete loss of basophilia. Histochemical studies showed that the cell is abnormally loaded with glycogen. Quantitative chemical determination on slices of liver of such animals showed a four-fold increase of glycogen as compared to that in controls.

71. Investigations into the carrier state of the virus of infectious hepatitis under Dr. B.K. Aikat at the S.S.K.M. Hospital, Calcutta.

The object of the enquiry was to investigate into the problems of :

1. Identification of :
 - (a) Carriers of the virus of infectious hepatitis in certain selected groups of population.
 - (b) Carriers in mothers of infants suffering from cirrhosis.
2. Follow up study of cases of infectious hepatitis.
3. The relation, if any, between infectious hepatitis of adult cirrhosis.

In the absence of any available methods of isolation of the virus, and the impracticability of undertaking human transmission experiments, the approach to the problem had to be indirect.

Stokes *et al* (1954), Necfe *et al* (1952), Fitch *et al* (1955) and Reinhold *et al* (1955) suggested the possibility of screening active carriers by intelligent use of a battery of liver function tests. The limitations of this method are obvious. There could be active carriers without any

obvious functional derangement and persons showing one or more abnormal functional tests may have hepatic pathology without being active carriers of the virus.

The presence of active silent carriers could not be denied.

Possibilities of undertaking other tests were thought of. On the suggestions of Dr. Work a complement fixation test using the sera in the acute early stages of the disease was tried. The results of these tests were not the least encouraging and were abandoned after being performed with convalescent and 25 control cases.

The investigations consisted of :

1. Good clinical record according to the proforma circulated with the last report.

2. Liver function test.

Total protein. Albumin and globulin serum-bilirium, alkaline phosphatase, thymol turbidity, Zn sulph turbidity and cephalin cholesterol, flocculation test, and electrophoretic pattern of serum proteins by moving boundary technique.

3. Liver biopsy in selected cases showing abnormal functions.

In the analysis of the first group of 400 cases it was indicated that in the final assessment, it would be desirable to group the tests found abnormal in positive control cases.

It was decided to 'score', the abnormalities on the findings of Zn sulph and thymol turbidity along with the results of electrophoretic pattern.

It has not been possible to submit the results of the 1210 cases investigated so far to a proper statistical analysis of the many variable factors.

The present report is a factual presentation of the available data.

The abnormal values have been taken to be those above the standard laid down by the diagnostic Lab., as indicated in the previous report.

In the final analysis, the abnormal values will be taken as those above twice the standard deviations after statistical analysis of the results of each group.

Abnormal results were repeated at least once

TABLE I

No. of cases investigated in different groups :

1. Antinatal and post natal mothers	252
2. Normal healthy women between the age of 18-40 years	188
3. Miscellaneous group from hospital cases (males) without any hepatic disease.	135

4. Blood donors	250
5. Mothers of established cases of infantile cirrhosis	78
6. Mothers of cases of marasmus	35
7. Infectious hepatitis	160
8. Follow up cases	56
9. Established cases of adult cirrhosis	52
Total	1206

TABLE II

Group	No.	Two test +	Three test +
Normal healthy women	188	28	18
Anti and post natal mothers	252	31	24
Mothers of infantile cirrhosis	78	24	20
Mothers of marasmic children	35	6	2

Liver biopsy was performed in six	
of the mothers of infantile hepatitis	2
Portal scarring with increase of infiltration	1
Normal	2
No opinion	1

TABLE III

Incidence of Jaundice in the siblings of antenatal mothers

		No. of jaundice
Normal hepatic function	... 216	6
Abnormal hepatic function	... 36 (2 test)	4
Abnormal hepatic function	... 24 (2 test)	4

TABLE IV

Incidence of Jaundice in neonatal mothers

		No. of jaundice
No. of cases	... 216	14
No. of cases	... 36	1

All the ten cases of jaundice in neonatal period have been followed up. Clinically there has been no indication of ill health so far.

TABLE V

Group	No. of cases	2 test +	3 test +
Miscellaneous group (Hospital male cases)	135	18	12
Blood donors	250	56	29

Biopsy performed only on four donors

Smoulding active hepatitis	...	2
Frank cirrhosis	...	1
Normal	...	1

7 cases developed jaundice 38-56 days after transfusion. These cases were diagnosed on clinical, biochemical and on liver biopsy as cases of infectious hepatitis. These donors could not be traced from the central blood bank. The blood bank however were rejecting donors due to presence of jaundice in the professional donors.

TABLE VI

No. of cases biopsied	...	160
Followed up by liver function	...	56
Followed up by liver function and biopsy	...	9

16 out of 56 cases showed abnormal function upto 9 months after the onset. 9 continued to show abnormal function and structures and became frank cases of cirrhosis in 24 months.

TABLE VII

Cases under different groups

Group I	Definite history of infectious hepatitis	...	11
Group II	History of jaundice	...	9
Group III	Without any history of jaundice	...	31
Total		...	51

TABLE VIII

Socio-economic status in each group

	No. of case	Upper	Middle	Lower Middle	Poor
Group I	11	1	2	3	5
Group II	9	...	...	3	6
Group III	31	2	2	8	19
Total	51	3	4	14	30

TABLE IX

Anatomical types of lesions in each group

	No. in each group.	Diff. Hep. Fibrosis.	Post. Nec. Cirrhosis.	Post. Nec. Scarring.
Group I ...	11	3	6	2
Group II ...	9	2	6	1
Group III				
(a) Biopsy ...	16	9	7	0
(b) Biopsy & Autopsy ...	4	1	3	...
(c) Autopsy ...	11	8	3	0
TOTAL	51	23	25	3

The data given above offers certain suggestive indication.

A more careful analysis will be necessary before any conclusions can be arrived.

72. Investigations to find out the biochemical changes and effect of different types of therapy in hepatic coma under Dr. N. R. Konar at the N.R.S. Medical College, Calcutta.

Seventy-four cases of hepatic coma have been studied. The primary diseases of the liver which gave rise to hepatic coma were: Portal cirrhosis of liver 23, post-hepatitis cirrhosis 7, biliary cirrhosis 3, splenomegalic cirrhosis 4, fulminant hepatitis 8, subacute hepatic necrosis 7, acute infective hepatitis 13, carcinoma liver 4, cholangio-hepatitis 1, poisoning with copper sulphate 1, and with sulphone 1, Weil's disease 1, blood transfusion reaction in a case of Cooley's anæmia 1. Precipitating factors of hepatic coma (in 24 cases) were: Hæmatemesis and/or melaena 14, paracentesis abdominis 7, gastro enteritis 1, surgery (laminectomy) 1, blood transfusion 1. Clinical features of the cases when they were first seen were: (A) Mental—grade I—(slightest alteration of mental function) 8 cases; grade II (severe mental confusion and disorientation) 18; grade III (stupor and semicoma) 30; grade IV (coma) 19. (B) Neurological—(i) exaggerated deep reflexes—with extensor plantar and ankle clonus 6 cases,—with flexor plantar and ankle clonus 9,—with flexor plantar and no ankle clonus 8,—with extensor plantar and no clonus 9,—with no extensor plantar and no ankle clonus 3,—with no extensor plantar and clonus 5; (ii) absent deep reflexes—with no plantar reflex and no clonus 6,—with flexor plantar and no clonus 4,—with extensor plantar and no clonus 9,—with no plantar reflex and clonus 1,—with flexor plantar and clonus 1; (iii) normal deep reflexes—with flexor plantar and no clonus 8,—extensor plantar and no clonus 5. Flapping tremor was present in 4 cases. Hepatic foetor could not be detected in any of the cases. Disorders of consciousness, personality changes and disorders of the motor system were the important clinical manifestations. The changed mental state, flapping tremor, and signs of involvement of the pyramidal tracts were the most important features for the early detection of hepatic coma in a patient suffering from hepatic disease. The commonest conditions that gave rise to hepatic coma were portal cirrhosis of liver and infective hepatitis. Gastro-intestinal hæmorrhage was the commonest precipitating factor. The early recognition and energetic treatment of the precipitating factor was found to be of help in reversing coma, particularly in cirrhotic patients with reasonably compensated liver.

Urea-nitrogen was up to 20 mg./100 c.c. in 19 cases of which coma was reversed in 7, while 12 died,—20.1—50 mg./100 c.c. in 38 of which coma reversed in 6, while 32 died,—above 50 mg./100 c.c. in 17 of which coma reversed in 3, while 14 died. Non-protein nitrogen was upto 40 mg./100 c.c. in 30 cases of whom coma reversed in 7 cases, while 23 died,—40.1-80 mg./100 c.c. in 30 of whom coma reversed in 4, while 26 died,—above 80 mg./100 c.c. in 8 of whom coma reversed in 3, while 5 died. Undetermined nitrogen (mostly amino acids) was—up to 10 mg./100 c.c. in 15 cases of whom coma reversed in 5, while 12 died,—above 10 in 53 of whom coma reversed in 9, while 44 died. Serum bilirubin was up to 2 mg./100 c.c. in 11 cases of whom coma reversed in 7, while 4 died,—2.1-5 mg./100 c.c. in 30 of whom coma reversed in 5, while 25 died,—5.1-8 mg./100 c.c. in 21 of whom coma reversed in 4, while 17 died,—above 8 mg./100 c.c. in 12 of whom all died. Total serum protein was 0.6 g./100 c.c. in 30 cases of whom coma reversed in 7, while 23 died,—above 6 g./100 c.c.

in 38 of whom coma reversed in 8, while 30 died. Serum albumin was —0.2 g./100 c.c. in 13 cases of whom coma reversed in 3, while 10 died, —2.1-3 g./100 c.c. in 40 of whom coma reversed in 9, while 31 died, —3.1-4.5 g./100 c.c. in 15 of whom coma reversed in 2, while 13 died. Serum globulin was —1.6-2.5 g./100 c.c. in 9 cases of whom coma reversed in 1, while 8 died, —2.6-3.5 g./100 c.c. in 20 of whom coma reversed in 7, while 13 died, —above 3.5 g./100 c.c. in 41 of whom coma reversed in 6, while 35 died. Albumin globulin ratio was below 1 in 58 cases of whom coma was reversed in 10, while 48 died, —above 1 in 10 cases of whom coma reversed in 4, while 6 died. Serum cholesterol was up to 80 mg./100 c.c. in 28 cases of whom coma reversed in 4, while 24 died, —81-150 mg./100 c.c. in 32 of whom coma reversed in 7, while 25 died, —above 150 mg./100 c.c. in 9 of whom coma reversed in 5, while 4 died. Serum alkaline phosphatase (King Armstrong units) was 3-13 units in 13 cases of whom coma reversed in 5, while 8 died, —13.1-30 units in 15 of whom coma reversed in 3, while 12 died, —above 30 units in 10 of whom coma reversed in 3, while 7 died. Thymol turbidity (Maclagan units) was —0.4 units in 2 cases of whom coma reversed in 1, while 1 died, —4.1-6 units in 22 of whom coma reversed in 10, while 12 died, —above 6 units in 19 of whom coma reversed in 3, while 16 died. Cephalin cholesterol flocculation test was —+++ in 9 cases of whom coma reversed in 3, while 6 died, —++++ in 8 of whom coma reversed in 1, while 7 died, —+++++ in 1 who died. Zinc sulphate turbidity was —4-12 units in 1 case in whom coma was reversed, —12.1-20 units in 10 of whom coma reversed in 3, while 7 died, —above 20 units in 6 of whom coma reversed in 1, while 5 died. Blood chlorides was below 450 mg./100 c.c. in 45 cases of whom coma reversed in 5, while 40 died, —above 450 mg./100 c.c. in 29 of whom coma reversed in 11, while 18 died. Blood ammonia was —1-2.5 mcg/c.c. in 2 cases of whom coma was reversed in 1 case while the other died, —2.6-5 mcg/c.c. in 9 of whom coma reversed in 8, while 1 died, —5.1-7.5 mcg/c.c. in 11 of whom coma reversed in 4, while 7 died, —7.6-10 mcg/c.c. in 6 all of whom died, —10.1-15 mcg/c.c. in 6 all of whom died, —15.1-25 mcg/c.c. in 2 of whom coma reversed in 1 while the other died, —20.1-31 mcg/c.c., in 4 all of whom died.

Blood ammonia was determined by Conway's micro-diffusion method in the department of Prof. B.K. Aikat, M.D., Ph.D., Director, Department of Pathology, Institute of Post Graduate, Medical Education and Research at S.S.K.M. Hospital, Calcutta.

The liver-function tests studied showed significant derangement of hepatic function in hepatic coma but the degree of derangement was of no help in predicting or measuring the grade of hepatic coma. Although severe liver damage was a pre-requisite for the development of hepatic coma, no consistent correlation between changes in liver function tests and development of coma was evident. Rise in serum bilirubin level and fall in serum cholesterol level, however, had bad prognostic significance. The non-protein nitrogenous constituents of blood were significantly elevated in comatose state compared to precomatose state. Elevated blood ammonia level was the most constant biochemical abnormality in hepatic coma but there was no definite correlation between rise of blood ammonia level and depth of coma. Blood ammonia values also failed to correlate with the degree of hepatic dysfunction as judged by liver-function tests

and the depth of coma. Blood-sugar level was within normal limits in all cases except one. Blood-chlorides were lowered in 45 out of 74 cases of hepatic coma. Marked lowering of blood-chloride and serum cholestrol was found to be of grave prognostic significance. Cerebrospinal fluid also showed low chloride values. Bilirubin was detected in cerebrospinal fluid in 5 out of 47 cases of hepatic coma.

Liver tissue was taken out in 37 cases who died of coma by Vim-silverman's needle immediately after death. Histological changes were : (i) Degeneration of liver cells and plenty round cell infiltration with occasional polymorphonuclear infiltration into the portal space and extending into the lobules—with bile thrombi in 3 cases and without bile thrombi in 7 cases ; (ii) degeneration of liver cells and regeneration of some cells and some increase in fibrous tissue in portal area and round cell infiltration—with bile thrombi in 3 cases and without bile thrombi in 3 cases ; (iii) disorganisation of hepatic architecture and marked increase in connective tissue in portal tracts and few round cell infiltration and some regeneration of liver cells—without bile thrombi in 3 cases and with bile thrombi in 2 cases ; (iv) disorganization of hepatic architecture and portal fibrosis and proliferation of bile canaliculi and fatty change in 11 cases ; (v) malignant cells in 4 cases ; and (vi) swollen liver cells—sinusoids appear to be occluded and hydropic degeneration of some liver cells and round cell infiltration in portal area and bile thrombi in 1 case. The liver tissue histology revealed the underlying hepatic pathology but no change specific for hepatic coma was detected. No definite correlation could be found out between the structural alteration of the liver and the liver-function tests.

Therapeutic study was conducted on the patients on controlled regimes. Control group consisting of 21 patients was treated with supportive measures only, which included treatment of precipitating factor, if any, maintenance of calories, fluid and electrolyte balance, withholding of nitrogen intake either in food or in drugs and administration of vitamins. The test group consisting of 53 patients was treated with broad spectrum antibiotics, intravenous sodium glutamate and hydrocortisone in addition to the supportive measures. Three per cent aqueous solution of sodium glutamate was given intravenously to all the cases of the test series except in 1 case—16 g. of sodium glutamate was given at a time—once in 24 hours. Of the broad spectrum antibiotics, achromycin was given orally to 14 patients, aureomycin to 2 patients, mysteclin to 1 patient and terramycin to 27 patients. The antibiotics were given by nasal tube and the dose was 250 mg. every 6 hours. Tablets of cortisone or hydrocortisone of 25 mg. and 20 mg. each were given every 6 hours through nasal tube. In the control group were included 21 patients—5 of acute hepatitis, 2 of subacute hepatitis, 11 of cirrhosis and 3 of carcinoma liver. In the test group were 53 patients of whom 18 were of acute hepatitis, 5 of subacute hepatitis, 28 of cirrhosis, 1 of carcinoma liver and 1 of Cooley's anæmia. In the control group of 21 patients, only 2 cases survived from coma and both of them were cases of cirrhosis liver. In the test series of 53 cases, in 14 patients coma was reversed of whom 9 were from the cirrhosis group, 3 from acute hepatitis group, 1 was a case of carcinoma liver and 1 of Cooley's anæmia. The treatment was of no value in any of the 7

cases of hepatic coma following sub-acute hepatitis. Only 3 patients out of 21 of acute hepatitis who developed hepatic coma survived. Treatment was of some value in the cirrhosis group. Of the 35 patients in this group 11 recovered from coma of whom 3 survived for more than 6 months. Sodium glutamate lowered the blood ammonia level significantly but did not restore it to normal. The lowering of blood ammonia level was not always accompanied by improvement of mental state. Immediate prognosis was better in hepatic coma due to cirrhosis than in those due to acute and subacute hepatitis. The long term prognosis depended on the extent of hepatic reserve. In the 3 patients of acute hepatitis who recovered from coma, the recovery was complete.

73. Inquiry on blood and CSF ammonia and glutathione in liver disease, with or without coma and the effect of glutamate on these levels and on the clinical condition under Dr. Shiv Kumar at the Medical College, Amritsar.

Blood and CSF ammonia and GSH concentrations have been determined in 42 cases of liver disease, 13 of which were in coma. In addition, these values were also determined in 6 cases of coma other than hepatic.

Blood NH_3 concentration was, generally, raised in liver disease, except infective hepatitis, and in cases of hepatic coma. No significant relationship has, however, been observed between the blood ammonia levels and the clinical status in the cases studied.

After administration of glutamate intravenously, immediately following the transfusion, blood ammonia concentration registered a slight fall which was temporary. In fact, as the transfusion was continued, ammonia concentration continued to rise. Coma was in no case terminated by this treatment. Ammonia tolerance studies were done in 10 normal adults and in 15 cases of hepatic disease.

74. Inquiry on electrophoretic and chromatographic studies in experimental animals and human beings in health and liver injury under Dr. Balasubrahmanyam and Dr. Ramji Dass at the Government Medical College, Patiala.

The object of the work was to study the serum electrophoretic patterns and the amino-acid content of serum and liver tissue in human cases of liver disease and in experimentally produced liver injury.

Electrophoresis of serum proteins was carried out on filter paper in a horizontal position, using berate barbitone buffer of pH 8.6.

For rat sera, a phosphate buffer of pH 8 was used. The stained strips of papers were scanned under a photo-electric densitometer and curves plotted. The concentration of the individual fractions of proteins were calculated by measuring the areas under the curves with a planimeter. Thirty-five cases of cirrhosis of liver and 18 cases of infective hepatitis were investigated. Most of the cases of cirrhosis showed a marked increase of the gamma globulin fraction with a corresponding

reduction in the other globulin and albumin fractions. About half the number of cases showed a characteristic merging of the beta and gamma fractions of globulin, so that their separation was poor. The electrophoretic pattern had a fair correlation with the liver-function tests as thymol turbidity but no quantitative correlation could be established between the pattern and the severity of liver damage as judged by the needle biopsy. In infective hepatitis cases, with a short history, the pattern was normal, while in those of longer duration the gamma globulin was increased.

Amino-acid analysis of serum was carried out by single dimension chromatography, using concentrated extracts from serum after precipitating the proteins with acetone. The chromatograms were scanned under a photo-electric densitometer and the percentage of each amino acid in relation to the total amino acids was calculated. In six cases the glutamic acid content was raised above normal. There was no significant alteration in others. There was no obvious correlation between the amino-acids content and the electrophoretic patterns.

The free amino acids in the liver were analysed by double dimension paper chromatography after preparing concentrated extracts of the tissue.

75. Study of the liver in cirrhosis following ligation of the splenic artery under Dr. F.P. Antia at the Topiwala National Medical College, Bombay.

Seventeen cases of cirrhosis of the liver were investigated. Three underwent splenic artery ligation of which two improved as seen clinically and by liver-function tests, third is still in the ward. Three cases had to undergo splenectomy as the spleen was considerably enlarged (one of these was Curveilher Baumgarten syndrome). One died of hepatic coma on the third day of operation. Two cases in children were considered early cirrhosis and are under observation with better nutrition. Three cases are investigated and are advised operation. One case has refused operation. Five cases expired, two with hæmetemesis and three due to extreme malnutrition.

76. Investigation into some aspects of pathogenesis of ascites with special reference to the ascites in cirrhosis under Dr. N.C. Nayak and Dr. G.S. Mohapatra at the S.C.B. Medical College, Cuttack.

During this period 15 cases of ascites have been investigated. Liver biopsy, liver-function tests and examination of ascitic fluid were done in all patients. Five patients with cirrhosis of liver were subjected to radiological examination for oesophageal varices. Punch biopsy of liver was done by Vim-Silverman needle. Among the hepatic-function tests, serum protein estimations (total, albumin and globulin) by micro-Kjeldahl method, serum bilirubin, thymol and zinc sulphate turbidity tests, thymol flocculation and alkaline phosphatase estimations were carried out. Out of 15 cases, clinically 13 were cirrhosis, one was a

case of hepatoma and another a case of hepatosplenomegaly. In cirrhotic cases the albumin level was usually low, the zinc sulphate turbidity showed high unit value and thymol turbidity was also raised. Few cases showed slight increase in serum bilirubin content. The hepatoma case showed on biopsy a combination of liver-cell carcinoma and cholangioma. Two cases of clinically diagnosed cirrhosis which showed on biopsy very early fibrosis or a normal liver structure, improved noticeably after hospitalization and paracentesis. None of the 5 cases X-rayed for oesophageal varices showed evidence of any portal hypertension.

77. "Clinico-pathological study of liver in asphyxiated bates (still birth) and dead infants under Dr. Nawal Kishore and Dr .P.N. Wahi at the S.N. Medical College, Agra.

The work was started with a view to study infants born dead or dying soon after birth due to oxygen to find out if the infant's liver and other organs show some specific pathologic findings and to correlate such findings with further studies on the mothers of these infants. We have done 12 cases till date (within the last three months). The collection of material has now been extended to the Lady Lyall Hospital, Agra, and Janki Devi Hospital and it is hoped that more cases would be available.

We have reached a position in which we could start the second phase of our objectives, i.e., colateral studies of the mothers whose babies show liver pathology.

We have also plans to follow such infants who are born asphyxiated and whom we are able to revive. The period of anoxia before being revived may cause early injury and may cause such damage which later may predispose to liver pathology or liver cirrhosis. Such infants would be followed up 3 monthly up to the age of 3 years, to find out if they have pre-disposition to liver pathology as contemplated. This would also be evaluated in relation to the time factor of asphyxia for the initial damage would be proportionate to the time the baby was asphyxiated.

78. Inquiry into the effects of crude liver extract used intravenously in experimental hepatic cirrhosis under Dr. L.R. Sarin and Dr. J.C. Sarin at the S.M.S. Medical College, Jaipur.

Plasma total proteins, albumin and globulin content, serum bilirubin, thymol turbidity, thymol flocculation and direct van den Bergh's tests were carried out in 50 dogs, caught at random. The plasma-protein values varied from 6.2 g. to 7.3 g. per cent and the albumin to globulin ratio ranged from 1.0 : 1.0 to 2.5 : 1.0. Thymol-flocculation test was negative in all the animals and thymol turbidity varied from 0 to 2 units. Values for serum bilirubin were 0.1 mg. to 1.0 mg. per cent and direct van den Bergh's test was negative in all the animals tested.

Microscopic anatomy of the liver in 10 of the above dogs was studied by liver biopsy.

In order to work out a method for producing cirrhosis in dogs with carb on tetrachloride, healthy male puppies varying in age from 3 month

to 8 months, were selected for the investigation. A preliminary liver biopsy and liver-function tests were carried out in each and only those animals in whom these tests were found normal were used for further experiments. The animals were fed on 2 to 4 chattacks of wheat, a pao of milk and half a chattack of boiled meat with added salt. Carbon tetrachloride was administered through various routes.

The results of these various procedures used were as follows :—

(a) *Daily administration of carbon tetrachloride by inhalation* :—In a concentration of 1 : 5000 was tried in 4 dogs. All the animals died within a period of one and a-half-month, two because of hæmorrhagic necrosis of liver, one of nephrosis, and the remaining one of pneumonia.

The procedure was not investigated further.

(b) *Subcutaneous administration of CCl_4 mixed with liquid paraffin* by multiple punctures, lead to sterile abscess formation in the two animals used for this purpose. The procedure was considered undesirable for the aim in view.

(c) *Oral administration of carbon tetrachloride*.—Attempts to feed orally CCl_4 in gelatin capsules, in emulsion form, in milk were made but failed.

(d) *Administration of CCl_4 by intragastric route*. It was carried out with the results as below :—

(i) *2.5 c.c. of CCl_4 every 4th day*, as a first thing, in the morning, was given to 5 dogs. A month later liver function tests and liver biopsy in one dog revealed no change. Another dog who died (after 19 such administrations) because of CCl_4 entering lungs, showed slight central necrosis of liver and no other change.

As a consequence to above, the administration of carbon-tetrachloride to remaining dogs was changed over to administration on alternate days.

(ii) *2.5 c.c. of CCl_4 every alternate day to dogs in which previously CCl_4 was given on 4th day*.—Two of these dogs died after 4 to 5 administrations on alternate days and autopsy revealed focal necrosis and fatty degeneration (one dog) and peripheral necrosis of liver in the other, respectively.

In another dog, which was given 7 intragastric administration on every 4th day, followed by 22 administrations on alternate days (in approximately 13 weeks) hepatic function showed deterioration. After further seven such administrations, liver biopsy revealed a picture indistinguishable from that of cirrhosis liver. Ten days after liver biopsy, the animal died of gas gangrene liver.

Another dog in this series, after 13 every 4th day administrations and 30 alternate day administrations, showed impairment of liver function tests and on liver biopsy fatty degeneration, focal necrosis and slight

but irregularly distributed fibrosis. Administration of carbon tetrachloride was left at this stage. A month later liver-function test showed improvement but the histological picture of liver was the same. Six further alternate day administration of carbon tetrachloride were given. This resulted in impairment of hepatic function. The dog started getting emaciated, carbon tetrachloride administration was given up. A month later the dog died. At autopsy liver revealed changes which were diagnosed as early cirrhosis.

(iii) *Intragastric administration of CCl_4 on every alternate day to 10 dogs* was started. All the animals died within a period of 45 days of the start of the experiment.

A dog dying after 5 administrations showed fairly marked central necrosis and slight fibro-blastic proliferation and infiltration with polymorpho-nuclears. Four dogs dying after 7,7,8 and 9 such administrations, respectively, showed extension of necrosis toward periphery, increasing congestion of sinusoids and central veins, increased fibro-blastic activity and some bands of fibrosis and replacement of polymorpho-nuclear by plasma cells, lymphocytes and macrophages, and infiltration of portal tracts with same cells. Some of the portal tracts showed more than one bile duct.

Five dogs who had been given 11,11,12,12 and 23 intragastric administrations, respectively, died of gasgangrene liver. Histological examination revealed total loss of structure except in two dogs and infiltration with rod shaped bacilli. In two dogs (11 administration each) the gasgangrene was not such advanced and in these, less of normal lobular architecture, hyperplasia of liver cells, denser and increased bands of fibrosis, marked congestion of sinusoids and infiltration of portal tracts with the plasma cells and lymphocyte was seen. Bands of fibrous tissue extended from portal tracts into liver perenchyma.

79. Inquiry on the role of vitamin E and molybdenum in liver disorders under Dr. N.G. Magar at the Institute of Science, Bombay and Dr. T.P. Bhardwaj at the Topiwala National Medical College, Bombay.

1. *Phospholipids of blood, brain, kidney and liver.*—The effect of molybdenum and vitamin E on the phospholipids of the blood, brain, kidney and liver were studied on the animals maintained on control, molybdenum 100 ppm and molybdenum 100 ppm plus vitamin E, 8 mg. per rat per week. The experimental animals were sacrificed after two months on the experimental dietary, by means of bleeding by cardiac puncture. Analytical data revealed that whereas lecithin and sphingomyelin were constant, in all the groups, cephalin recorded a rise in the molybdenum-fed group. The mechanism of this phenomenon has yet to be elucidated. In all cases, vitamin E prevented the changes brought about by molybdenum.

2. An initial experiment is in progress to establish the toxicity, if any, of molybdenum on the hepatic system and to demonstrate the effect of vitamin E on the changes thus induced. Albino rats have been

maintained on a standard casein diet (18 per cent) with supplementation of molybdenum at 100 ppm in two groups, with and without supplementation of vitamin E. Towards the end of the experimental period it is proposed to examine the livers for structural and biochemical changes.

3. A second batch of albino rats have been placed on experiment to determine the metabolism of vitamin E and molybdenum in hepatomas induced by butter yellow. Inbred strains albino rats have been distributed into an orientation with multiple dietary regimen. They are intended to be placed soon on different dietary regimen consisting of molybdenum *plus* butter yellow; molybdenum *plus* butter yellow. The average survival time, average duration for hepatic injury to set in and percentage fatalities will be determined to study whether molybdenum hastens the hepatic damage caused by butter yellow.

The livers of animals in both the above experiments will be assayed for xanthine oxidase activity by the Warburg microrespirometer. In addition protein N, nucleic acids, and electrophoretic pattern of liver proteins will be studied.

COMMUNICABLE DISEASES

CHOLERA

80. Cholera inquiry under Dr. K. Bhaskaran at the Central Drug Research Institute, Lucknow.

Genetic Studies.—Attempts to show the occurrence of genetic exchange in *Vibrio cholerae* proved successful with strains Ogawa 121/s and Inaba C₄. These strains are sensitive to phage 129 (associated with lysogenic strain of *V. cholerae*, R 129) and when cultivated with it yielded a significantly large number of colonies with altered characters. For instance, in one such 'cross' between strain R 129 (purine-requiring, streptomycin-sensitive, Rough) and 121/s (methionine-requiring, streptomycin-resistant, Smooth Ogawa type), 600 colonies appeared on a solid minimal medium (devoid of purine and methionine), while controls with equivalent populations (10⁹ cells) of each culture yielded only 36 and 12 colonies respectively, which indicated their frequency of mutation to prototrophy or non-exacting condition. Prototrophic colonies isolated from the pool had the unselected markers of the parent strain 121/s in that they were streptomycin-resistant and smooth (Ogawa type). All of them were lysogenic for phage 129. The possibility that this phage may have played a role in the transfer of methionine independence to 121/s was further strengthened when strains Inaba 6, Inaba 20, and Ogawa 23, lysogenised with phage 129, were utilised for crosses with 121/s. Here again transfer of methionine-independence could be demonstrated unequivocally, which was not evident when the parent non-lysogenic strains were employed.

Similar results were obtained with a stable non-motile, streptomycin-resistant strain of *V. cholerae* (Inaba C₄). A large number of

motile cells of this strain appeared as 'flares' in gelatin-agar media, from a cross of this strain with R 129, or any other strain lysogenised with phage 129. This acquisition of motility by Inaba C₄, could not be demonstrated in the absence of phage 129.

These findings indicate that phage 129 is capable of transferring (transducing) some characters during its shift from host to host in a manner analogous to genetic transduction in *Salmonella*. However, it has not so far been possible to obtain high titre preparations of this phage, free of viable *V. cholerae*, which could be employed in such experiments to prove conclusively the nature of genetic exchange observed.

Experiments with other lysogenic strains of *V. cholerae* on similar lines, were however not successful indicating that not all phages are endowed with transducing property. Strains Ogawa 121/s and Inaba C₄ appear to be reliable indicators for the detection and isolation of transducing phages from lysogenic strains of *V. cholerae*.

Lysogenic strains of V. cholerae.—Further cross-matching tests in a collection of 127 strains of *V. cholerae* have shown that a vast majority of them are lysogenic, producing plaques on sensitive indicator strains. A variety of temperate phages have been identified, which are yet to be differentiated from one another by serological tests. Sensitivity tests of *V. cholerae* cultures to these different phages are being carried out. Already certain patterns have emerged, the significance of which is not yet clear. It is possible that some of these phages may be of value in typing *V. cholerae*.

81. Inquiry on the evaluation of phages acting on vibrios and the application of bacteriophage typing in epidemiological investigation on cholera under Dr. M.N. Lahiri and Dr. B. Ghosh Roy at the All-India Institute of Hygiene & Public Health, Calcutta.

The study has been undertaken to evolve a suitable method for typing strains of *V. cholerae* on the basis of the lytic pattern with different races of cholera phage. This may be helpful in epidemiological investigations to trace the source of infection and to plot its spread. The phages are also being studied to elucidate the inter-relationship between the cholera vibrios of Sub-group I and other inagglutinable vibrios isolated from patients and other sources.

Synopsis of work done.—Isolations of strains of *V. cholerae* have been made from the stools of cholera patients during the last cholera epidemic in the city; as many faecal specimens were collected on or about the 3rd and 4th day of illness of the disease, direct plating gave unsatisfactory results. Hence, modified Wilson & Blair's liquid medium pH 8.4 was used for enrichment. This medium yielded a higher percentage of positive isolations and gave good growth when incubation was continued beyond 24 hours. In fact, many samples which proved to be negative after 24 hours, were found positive after 48 to 72 hours of incubation. From certain cases associated N.A.G. vibrios were also isolated. These strains along with the strains isolated in previous years are being maintained in the laboratory for further studies.

Attempts to isolate phage from stools inoculated into Wilson & Blair's medium referred to above and used for the isolation of vibrio strains, yielded very disappointing results. Instead, for the purpose of isolation, nutrient broth pH 7.6 was used. For primary isolation, 'phage enrichment' method was adopted but the young vibrio culture which was added to the stool emulsions in broth was invariably prepared from the strain isolated from the same sample of stool. For the purposes of isolation of phages, this procedure proved very useful.

To study the morphology of plaques as well as to demonstrate their presence, nutrient agar from 1.2 to 1.5 per cent is being employed. The phages isolated during the period under report as well as those isolated earlier totalled 30 'pure line' strains. These phage preparations which are in stock are being kept in the laboratory at a temperature ranging from 0–4°C. and serial propagation is being carried out on the corresponding strain of *V. cholerae* at regular intervals and they are also being checked up to ensure their purity.

The critical test dilution was found out for each strain of phage and it was observed that at their optimal dilutions, no particular difference could be elicited with regard to the lytic pattern on both Inaba and Ogawa strains. Cross titration of the phage preparations at their optimal critical test dilution on the agglutinable strains of *V. cholerae* suggested some difference amongst the strains on the basis of the lytic pattern. With the phage preparations in hand, recent studies indicated that it is possible to divide the phage strains into five broad groups. Further trials with these groups of phage preparations are being carried out on more strains of *V. cholerae* collected from other laboratories to observe whether similar pattern of lysis could be obtained.

Studies to identify 'lysogenic' strains of *V. cholerae* were carried out and of the several strains examined, only six 'lysogenic' strains could be detected so far. When a few more strains are isolated, detailed studies of such strains will be undertaken.

Lyophilization of a few strains of phage did not yield uniform results. The work will be undertaken to study its effect on the stability during storage.

82. Cholera inquiry (endotoxin) under Dr. E.K. Narayanan at the Central Research Institute, Kasauli.

The inquiry was started in 1956-57 for elucidating the pathogenesis of cholera, particularly with reference to the endotoxin theory. Work done till September 1956, was to collect and store the materials for the research. Work was continued till April 1957 on critically examining the experimental methods of Burrows (1944) concerned with the endotoxin theory. The absence of reproducibility in permeability of normal membranes was noticed. The only practical method of demonstrating the permeability-enhancing property of the endotoxin was found to be the use of the membrane under test as its own control, after applying the necessary correction for change in permeability due to incubation. The average value of such a correction was determined. Investigations were

then continued to test Burrows' claim of a straight line plot for rate of flow of fluid through normal membranes. The claim could not be substantiated. Our result is supported by Poiseuille's equation. Work done from April to-date has been to further investigate the permeability test procedures, especially the form in which the vibrio cell material is to be used for the endotoxin test. Glycine lysates, urea solutions and chloroform lysates of vibrios were tried for their suitability for the permeability tests. The first two were found to be unsuitable because of the toxicity of glycine and urea to the membranes. So far, no objections have been found with regard to chloroform lysates. A procedure has been standardized for preparing such lysates with which the permeability factor of *V. cholerae* is proposed to be tested.

83. Immuno-chemical studies with reference to vibrio polysaccharides and proteins under Dr. Gurkirpal Singh and Dr. P. Devi at the Central Research Institute, Kasauli.

Polysaccharides were prepared from an El Tor vibrio and a rough strain of *V. cholerae* by phenol treatment. These polysaccharides had more or less similar chemical and immunological characteristics to that of those prepared previously from *V. cholerae* Inaba and non-agglutinable vibrio. A polysaccharide from *V. cholerae* Inaba 569B was also prepared by extraction with N/20 acetic acid. This polysaccharide had a much lower nitrogen and phosphorus content and high reducing substances compared to the above ones. Chromatography of acid hydrolysates of all these polysaccharides showed that a similar pattern of sugar constituents were present in all—ribose, a hexose, a uronic acid and a hexosamine.

In the course of the preparation of the polysaccharides by phenol treatment, it was found that the yields varied to a great extent from batch to batch and strain to strain. It was also observed that the 90 per cent phenol extract contained a good amount of water-extractable polysaccharides in solution which were precipitated on dilution of the extract with water. Modification of the phenol treatment were then tried, such as treatment with 90 per cent phenol only once instead of twice and treatment with 75 per cent phenol. The time of contact of the dried bacteria with phenol too was changed. These experiments showed that treatment with lower strengths of phenol and for shorter periods gave polysaccharides in greater yield with higher precipitin titre and slightly lower nitrogen and phosphorus content. As a result treatment with 75 per cent phenol for 8 hours, with intermittent shaking by mechanical means, has been adopted for the preparation of polysaccharides.

In order to find out if ribose present in the polysaccharide preparations was due to admixture or combination with nucleic acid, nucleoprotein fractions were prepared from smooth and rough cholera vibrios by extraction with 0.14M sodium chloride solution (Kerr and Scraidan, *Jour. Biol. Chem.*, 1949, **180**, p.1203). Chemical analysis of the different nucleoproteins showed that the preparations from the rough strains had higher phosphorus and pentose value compared to those from smooth strains. Also, the former had a lower precipitin titre than the latter.

The nucleoprotein fraction from the smooth strain was shown to produce protective antibodies against *V. cholerae* when injected into mice. Chromatograms of the acid hydrolysates of all these fractions showed only two bands indicating the presence of ribose and hexose.

The bacterial residues after extraction with sodium chloride were again treated with phenol and polysaccharides prepared. The polysaccharide from a smooth strain obtained in this manner, though not having a high precipitin titre, evoked in mice protective antibodies against lethal doses of *V. cholerae*. These preparations still had ribose present in them. Chromatograms of the polysaccharides from smooth strains gave five bands, while those of the rough strains showed only two bands. Ribose and the hexose were present in both.

Attempts were made to prepare the polysaccharides from bacterial deposits and supernatant centrifugate. The amount of bacteria obtained was too small to get any significant amount of polysaccharide. Preparations, which were Molisch positive and Biuret positive and which precipitated with precipitin serum raised against *V. cholerae*, were obtained by acidification of the culture medium, after centrifuging off the bacteria, to pH 4.2, removal of the precipitate formed, dissolving it in water adjusting the pH to 7 and precipitation of the soluble portion with alcohol. Larger amounts of this preparation are being prepared in order to purify it further and make a full chemical and immunological study of the same.

84. Inquiry on the bacteriological investigation of cases of cholera with a view to find out the endemicity of cholera in Calcutta under Dr. S.N. De, Dr. K.C. Basu Mallik and Dr. Mondal at the Medical College and Nilratan Sircar Medical College, Calcutta.

Of 6,003 cases admitted with choleric symptoms in the year, 2,039 specimens of stool or rectal swab have been cultured on McConkey's medium. From 359 cases *V. cholerae* was isolated, 295 being Ogawa type and 64 Inaba. The other 1,680 cases yielded *Bacterium coli* with or without other organisms, viz. atypical *B. coli*, *B. alkaligenes faecalis*, *Proteus morgan*, *B. dysentery*, *B. pyocyaneus* and non-agglutinating vibrio. None of the latter belonged serologically to group A. The samples collected within 6 hours of onset showed the maximum percentage of success in isolation of *V. cholerae*, this decreased with increase of the time of collection. The nature of material from which *V. cholerae* was isolated varied from rice water to faecal stool or rectal swab.

A technique for detection and titration of cholera O and H-agglutinin in the patient's serum has been devised. For O-agglutinin, 4 hours incubation of dilutions of the serum with living suspension of Ogawa organisms at 37°C. and for H-agglutinin 22 hours' incubation with formalised broth culture of a (group A) N.A.G. vibrio at 56°C. were found satisfactory. All the culture positive cases (including those due to Inaba types) showed a rise of titre of O-agglutinin varying from 1 in 25 to 1 in 2,500 in course of seven days except 4 cases which were moribund. H-agglutinin seemed to appear earlier in some cases but many 7 day sera were marked by its absence. Taking rise of O-agglutinin as an evidence

of infection by *V. cholerae*, 88 of 169 (52 per cent) of culture negative cases were thought to be true cases of cholera. None of the culture negative cases in which stool was collected within 6 hours of onset showed rise of titre of O-agglutinin.

Ninety-seven cases gave history of cholera inoculation within a year and of these 21 were culture positive. Sixteen culture positive cases were inoculated within 1 week before attack, 4 within 2 weeks and 1 within 3 months. Six culture negative cases showed rise of O-agglutinin and they were inoculated within one week. None of them showed any agglutinin in their first-day serum. Two culture negative cases inoculated 1 and 3 months before showed H and/or O-agglutinin up to 1 in 83 but neither of these became higher in 7 days.

There were only 3 weeks out of 63 between 1.7.56 and 15.9.57 when culture positive cases were missing. Every other week yielded at least one culture positive case coming either from Calcutta or its suburbs. In no police zone of Calcutta, the cases lingered for more than 7 weeks at a time—they tended to persist longer at Sealdah Station area among refugees, and at Tollygunj, Beliaghata and Khardah. There were some 20 cases in whom swallowing of Ganges water was followed by attack of cholera and who had no knowledge of contact with a case of cholera.

85. Immuno-chemical studies in *Vibrio cholerae* under Dr. D.L. Shrivastava at the Central Drug Research Institute, Lucknow.

During the last six months that this inquiry has been in operation, 8 samples of polysaccharides from Ogawa 368 and Inaba 368 were prepared. In order to avoid contamination of the polysaccharides with agar, the two strains were grown in papain digest meat broth (pH 8) for 72 hours, after which the cultures were phenolized and centrifuged in sharples. The centrifugate was concentrated ten-fold in cyclone evaporator and the polysaccharide was precipitated out with alcohol and purified (Cf. Shrivastava and Seal, Proc. Soc. Exp. Biol. Med., 36, 157, 1937). Last traces of impurities and protein were got rid of by centrifuging the end product at 25,000 rpm. The clear supernatant was lyophilized and a pure white substance, easily soluble in water was obtained. Working with 12 litres, yields of the polysaccharides were from 250 to 850 mgs. per batch.

Physical and chemical characteristics of the polysaccharide samples prepared above are being studied. Results obtained so far, if confirmed by later findings with a larger number of samples, would show the following two interesting differences between the Ogawa and the Inaba polysaccharides :-

- I. Ogawa polysaccharide has been found to be levo-rotatory whereas the Inaba one dextro-rotatory, the specific rotation being -36.6 to -76.6 and $+23.6$ to $+36.6$ respectively.
- II. The hydrolysates of the polysaccharides with N/1 hydrochloric acid, when analysed chromatographically on Whatman filter

paper No. 1, give four spots in the case of Ogawa and three in Inaba. Two of the three common spots are of galactose and arabinose respectively. The third spot occupying approximately the same position as ribose or glucuronic acid lactone has not yet been identified. The additional spot obtained in the case of Ogawa hydrolysate has a lower Rf value and is not yet characterised. It is possible that it may be that of an aldobionic acid.

86. Inquiry on production of experimental cholera with bacteria associated with cases of clinical cholera under Dr. S.N. De at the Medical College, Calcutta.

Six strains of *V. cholerae* maintained in the laboratory for 1 to 5 years, when injected as peptone water culture mixed with mucin into the small intestine of adult rabbits after ligature of part of their cæcum and after they had been treated with cortisone for three consecutive days, produced diarrhœa with fall of blood pressure and death of the animals.

Two of six strains of El Tor vibrios procured from Kasauli and from London, three of five N.A.G. vibrios isolated from cases of gastroenteritis and two sero-types (0-55, 0-111) of *Bacterium coli*—isolated from cases of gastroenteritis in London—behaved in the same way as *V. cholerae*. Four El Tor strains, two N.A.G. strains and four strains of *Bact. coli* from cases of gastroenteritis in adults failed to produce diarrhœa or death of the animals.

LEPROSY

87. Leprosy inquiry under Dr. N. Mukherjee at the School of Tropical Medicine, Calcutta.

1. Study with radio-active diamino-sulphenyl-sulphone tagged with S-35.—It has been possible to develop and standardize suitable methods for the tracer and auto-radiographic studies on the uptake of diamino-diphenyl-sulphone tagged with S-35 by leprosy patients.

After a single oral dose of 4µc/kg. of the tagged DDS, the concentration in the healthy tissue during the initial six hours was about 2 to 3 times less than that of the blood. The difference was generally made up at the end of 18 hours, when concentration of DDS in blood and healthy tissue was about the same. However, uptake of the drug by the affected tissue was about 10 times greater than that of the healthy tissue. When the daily rate of diminution of the concentration in blood is compared with the daily rate of excretion through the kidneys during this period, it is observed that the rate of disappearance from the blood is higher than the rate of clearance through kidneys. The higher concentration of the drug in leprosy tissue explains at least partially, the fate of extra quantity of the drug which disappears from blood but could not be accounted for in the urine.

After oral administration of the drug, the concentration in blood reached its maximum level in about six hours, but the total quantity of the drug in blood constituted only 8 per cent of the total dose and corresponding concentration being 0.6 mg. per cent. Had the drug passed directly from stomach to blood, the concentration should have been much higher as well as the rise quicker. It, therefore, seems probable that the drug is first localized in the cells of the gastro-intestinal tract from where it is slowly taken up by the blood.

Auto-radiographs of tissues show that the density of the radio-active grains was more marked in the affected than in the healthy ones. The localization of grains in affected tissues was more marked around hair follicles, sweat glands, in blood vessels and nerves. The density of grains was definitely higher in histiocytes, epithelioid cells and foamy cells. In affected nerves the grains were heavier and covered the whole of the affected zone. In bone marrow the megakaryocytes and metamyelocytes mainly show the presence of radio-active particles.

2. *Electron and phase microscopy of M. Leprae.*—The electron and phase-microscopic studies, in addition to confirming the findings of previous year, revealed a glea-like substance around almost all leprosy bacilli and particularly around globi. The bacilli from cases under sulphone treatment for about six months showed no significant variation in their morphological character. However, significant changes were noticed in the bacillary structures from cases having treatment for one year or more. The cell membrane showed ragged appearance, cytoplasm lost its consistency and pattern, mitochondria broke up into smaller granules, cell wall occasionally showed signs of distortion and absorption and glea matter was absent around these bacilli.

3. *Cytochemistry of M. Leprae.*—It has been possible to develop cytochemical methods for detection of bacillary lipids and their localization in the organism. The cell membrane, cell wall, and polar bodies showed presence of phosphatides. The concentration of phospholipids was higher in the polar bodies.

Changes in the cytochemical patterns of the Kedrowsky's bacillus treated *in vitro* with DDS were studied. The acid and alkaline-phosphatase reactions were increased at the beginning but became markedly less later on. DNA activity was always weaker. RNA gave stronger reaction. Metachromasia was increased. Hale reaction was increased during the later period. PAS gave faint reaction at the beginning and faded away gradually. Acid-fastness was markedly reduced during the later period.

4. *Immunochemistry of leprosy bacilli.*—So far 1,015 persons were simultaneously tested with a new antigen prepared from Kedrowsky's bacillus and Dharmendra's refined antigen. Of the 1,015 persons, 315 were lepromatous cases, 482 tuberculoid, 97 maculo-anæsthetic, 65 borderline, 27 indeterminate, 13 polyneuritic and remaining 16 persons had no sign of leprosy. The negative reactions obtained with the new and refined antigens in 307 and 306, respectively, out of 315 lepromatous cases confirm the findings of the previous year and indicate a close

similarity of the new antigen to the specific antigen from *M. lepræ*. A comparative study of the results obtained in other types of cases also revealed a marked correlation between the two antigens. As regards the late reaction it was always found to be negative in lepromatous cases. In some positive reactors, particularly of tuberculoid type, small papular induration up to a maximum of 3 millimeters on the third or fourth week was observed which decreased later on. Ten chemical fractions were isolated from Kedrowsky's bacillus of which only one has given uniform early skin reaction similar to the refined lepromin of Dharmendra. Histological study of a few early positive reactions revealed œdema and infiltration with polymorphs at 24 hours and at the end of 48 hours an infiltrate of pre-tuberculoid nature was observed.

5. *Histochemistry*.—Sections stained after Mc. Gee Russel's technique showed the presence of calcium in macrophages especially in the vacuolated or foamy ones. They also reacted positively to von Kossa's silver method. The micro-chemical test in them revealed presence of phosphate. Leprosy bacilli were found in large numbers within the macrophage cells where the organisms seem to thrive well, at the cost of the host cell. It appears that these cells become degenerated and converted into vacuolated cells containing lipoids, which is probably the end-product of this type of cytoplasmic degeneration. Histamine content of the lepromatous lesions was not significantly higher than that in the corresponding healthy tissues.

Section from tuberculoid lesions showed that some of the macrophage cells reacted positively to Sudan black B technique, indicating the presence of phosphatides in them. In reacting tuberculoid lesions, larger number of above type of cells were found to take up the stain, and the concentration of the stain was more marked in cells showing vacuolation in their cytoplasm.

88. Leprosy inquiry under Dr. V.R. Khanolkar at the Indian Cancer Research Centre, Bombay.

I. *A correlative clinical study of the early and late lepromin reaction*.—The lepromin reaction is a test that is employed frequently in the typing of leprosy. This reaction consists of the intracutaneous injection of an antigen usually prepared from lepromatous nodules and observations of early and late responses to this injection. While some authors have accepted the early reaction as significant, there are others who wait for the late reaction before interpreting the test. The positive early reaction shows itself within 24 to 48 hours as a zone of erythema and œdema. The late reaction is stated to be positive with the appearance in about three weeks at the site of injection of a nodule larger than 4 mm. in diameter, with a characteristic histological picture. The present inquiry was aimed to determine.

1. Whether there was any significant and constant correlation between the early and late lepromin reactions.

2. Whether such correlation was obtained better with one or other of the standard lepromins employed.

For the purpose of this study of the reactions, two types of lepromins have been compared. These lepromins were (1) lepromin prepared at Acworth Leprosy Home, hereafter referred to as ALH lepromin and (2) lepromin kindly supplied by Leprosy Research Department, School of Tropical Medicine, Calcutta, referred to as D lepromin. Fifty patients (35 tuberculoid and 15 lepromatous) of leprosy were included in these observations. Both early and late reactions were observed clinically and graded in accordance with criteria laid down by the W.H.O. Expert Committee on Leprosy. A few of the late reactions in the lepromatous group were subjected to histological examination.

Considering the tuberculoid cases alone, it was observed that the appearance of an early positive reaction correlated with a late positive reaction in 22 cases out of 35 with D lepromin, and 19 cases out of 35 with ALH lepromin. However, there was no significant difference in the total number of both early and late reactions, considered individually, with the use of either of these antigens.

In the lepromatous cases, the ALH lepromin behaved according to schedule giving doubtful or negative early reactions and negative late reactions in all the cases. On the other hand, with the D lepromin 5 out of 15 cases gave early positive reactions and 10 negative or doubtful early reactions. The late reactions in this group with the D lepromin consisted in the formation of nodules in 11 cases, and a clinically negative late reaction in the remaining 4 cases. Histological examination of 6 of the 8 larger nodules revealed a picture consistent with, or similar to late reactions in tuberculoid leprosy, in addition to the usual histology of the disease.

"On the basis of these findings, it was seen that there is a correlation between the early and the late lepromin reaction; this correlation is not quantitative. As there is a difference in the result of the early and late reaction in some cases, it is necessary for a final evaluation of any case of leprosy that both early and late reactions be carried out."

II. *A comparative clinical and histological study of the reaction to intradermal injections of lepromin supplied by Leprosy Research Department, School of Tropical Medicine, Calcutta and an antigen prepared from Kedrowsky's bacillus.*—It was reported last year that an antigen prepared from Kedrowsky's bacillus reacted on intradermal injection, in leprosy patients, in a manner similar to standard lepromins. As these findings appeared to be promising, particularly in respect of the Kedrowsky's antigen being able to replace lepromin, it was considered worthwhile to repeat the experiment in order to study the evolution of the reaction, both clinically and histologically, and to compare the findings with those elicited by intradermal injection of lepromin. Both the antigens (Kedrowsky's antigen, hereafter referred to as K and lepromin D) were obtained from the Leprosy Research Department, School of Tropical Medicine, Calcutta, through the courtesy of Dr. Mukherjee.

A total of 67 patients of leprosy (42 tuberculoid and 25 lepromatous) was included in these studies. 0.1 c.c. of D. lepromin was injected intradermally in one fore arm and the same quantity of K. antigen in the other. Clinical observations recording; and excision of the reaction for histological examination at the end of 24 hours, 72 hours, I week, II weeks and III weeks in groups of patients was performed. Both the early and late reactions were graded in accordance with the criterion followed earlier. (As in the foregoing study doubtful and negative reactions were classed together). Histological examination was performed on formalin fixed paraffin sections.

Considering the tuberculoid cases alone, it was seen that both clinically and histologically there was a marked parallelism between the responses obtained with K. antigen and D. lepromin at 24 and 72 hours and I, II and III week, respectively. As a rule, the reactions with K. antigen appeared to be more extensive than with a similar quantity of D. lepromin. The histological findings were essentially those of the positive lepromin reaction in both its early and late phases.

With the lepromatous cases, however, there were certain differences in the behaviour of these two antigens. There was a significant increase in the number of both early and late positive reactions with K. antigen over that obtained with D. lepromin. Thus, at the end of three weeks, 12 out of 12 cases that were available for observation gave significant positive reactions with K. antigen whereas only 6 out of 12 of these cases appeared to be positive with D. lepromin. Histological examination showed that all 12 of these K. reactions resulted in localized granuloma formation, 8 of them containing giant cells. With the D. lepromin, 4 of these 12 cases revealed no histological changes, 3 showed the picture of persistent early reactions and 5 attempted localized granuloma formation of which only one showed typical giant cells. In these five cases, ALH lepromin was given which gave no reaction at the end of the III week. "In view of the anomolous results with the K. antigen in lepromatous cases, we are of the opinion that it is not a desirable antigen for lepromin reaction."

III. A clinical study of the early and late reactions to the intradermal injection of the antigen prepared from normal human liver.—To test the validity of the claims made by Kooij and Gerritson that human liver tissue behaves in a manner similar to lepromin (in both its early and late reaction), four antigens with various sizes of particles and concentrations were prepared from human liver tissue following these authors method. ALH lepromin was used as the control antigen. The reactions were studied in 41 tuberculoid and 27 lepromatous patients respectively. Control reactions behaved as per schedule in both the polar type of leprosy. On the other hand the liver tissue antigens gave no reactions in all, except one case of tuberculoid leprosy where a nodule was formed in late reaction. Thus, negative reactions obtained by liver antigen in tuberculoid cases appear to refute the claim made by these authors that 'lepromin reaction is a foreign body reaction'.

89. Leprosy inquiry under Dr. Paul W. Brand, at the Christian Medical College, Vellore.

(i) *Surgery of the hand.*—No completely new operations have been added this year. Many-tailed graft reported last year, has been further developed, and a detailed follow-up assessment made on all cases.

(ii) *Face surgery.*—Total re-construction of the nose has been attempted on the lines of the ancient operation of rhino-plasty practised by the Hindus of Northern India many thousand years ago.

(iii) The study of the bio-mechanics of gait has led us to much better appreciation of the true ætiology of ulceration. At this early stage of our study, we have already been able to find a partial answer to the problem of ulceration by providing a fully moulded shoe, individually prepared for every patient, which is rigid from end to end and which rocks on a central pivot rather than being allowed to bend. The incidence of ulceration with this type of shoe is significantly less than the incidence with an ordinary moulded shoe, or with traditional forms of chappals, or with bare feet.

It is our intention, to carry out more precise studies with an accurate pressure-sensing element imbedded in the shoes of normal and ulcerated patients. This pressure-sensing element will be recorded by a Cathode Ray Oscilloscope and will give us an accurate estimate of the success or failure of different types of shoe in spreading the strain of weight-bearing and the thrust of locomotion equally all over the foot. The area of the foot which is liable to ulceration should be spared to any strain at all, and unulcerated skin from the instep and sides of the foot may be recruited to share the strain instead.

90. Leprosy inquiry under Dr. N. Figueredo at the Acworth Leprosy Home, Bombay.

I. RESULTS OF EXAMINATION OF CONTACTS

(a) Examination of 364 family members (contacts) of patients revealed that 49.45 per cent were uninfected, 16.48 per cent were in the infected stage, 10.72 per cent had the earliest lesions and 23.35 per cent had already contracted the disease.

(b) *Lepromin test* :—

1. The test was positive in 35.55 per cent and negative in 64.45 per cent of the uninfected contacts.
2. The test was positive in 87.34 per cent and negative in 11.66 per cent of the infected (without lesions) contacts.
3. The test was positive in all those with early lesions, except in one child with presistent lesion-early neural, whose late reaction measured only 1 mm.

(c) *Development of lesions.*—The results of observation and examination of uninfected and infected contacts and of those with the earliest lesions examined in previous years revealed the following :—

1. 25 per cent of the uninfected contacts got infected.
2. 10 per cent of the uninfected and 15 per cent of the infected contacts developed primary lesions.
3. None of the uninfected, 35 percent of the infected, and 12·5 per cent of those with primary lesions developed macules with sensory impairment.
4. 65 per cent of the uninfected contacts remained uninfected.
5. 23·35 per cent of the infected contacts remained infected.
6. 12·5 per cent of those with primary lesions remained static.

(d) *Regression of lesions without treatment* :—

1. 29·4 per cent of the infected contacts became negative for bacilli but the lepromin test was still positive.
(Period : Minimum 2 years 8 months ; Maximum 4 years 9 months.)
2. The Primary lesion in 66 per cent vanished (period : Minimum 1 year; Maximum 3 years 10 months) in 50 per cent of those contacts in whom the disappearance of the lesions was noticed, the bacilli also disappeared.
(Period : Minimum 2 years 9 months; Maximum 3 years 10 months.)
3. In 1 out of 2 contacts with persistent lesion-early neural, the lesion vanished and the bacilli also disappeared in 3 years 8 months.

II. RESULTS OF EXAMINATION OF NON-LEPROSY CASES

A few acid-fast bacilli were found on the first examination in 6·36 per cent (7 out of 110) of persons examined on suspicion of leprosy, and who were not suffering from disease.

These results when compared to those obtained in case of contacts reveal a significant variation and provide additional support to the opinion that the acid-fast bacilli found in contacts are *M. lepræ*.

III. PROPHYLAXIS WITH DDS.

Dose schedule :

Contacts under 5 years	5 mg. DDS twice a week
„ from 5 to 10 years	10 mg. DDS twice a week
„ from 11 to 15 years	25 mg. DDS twice a week
„ over 15 years	50 mg. DDS twice a week

(A) *Detailed study with laboratory investigations.*—

One hundred contacts on regular prophylaxis have been observed during a period of 6 months to 3 years. The condition noted from time to time in these contacts is presented below, together with a comparable group of untreated contacts.

1. *Negative Contacts (uninfected).*—In the treated group, out of 47 negative contacts, 45 remained uninfected, and 2 became infected without developing any lesions; whereas in the control group, out of 65 negative contacts, 44 remained uninfected, 19 became infected, one developed primary lesions and one developed persistent lesions—early neural.

Follow-up of negative children contacts over an extended period has shown that those who got infected do so in a minimum period of 4 months and a maximum period of 3 years 5 months with an average of 1 year 3 months. Of the above 47 treated contacts, 20 children had been followed up for a period of 1 to 2 years. Of these 20, 2 became infected. In a comparable group of 31 (out of the untreated 65 above) children, 12 became infected and one developed primary lesions during the same period of observation.

The average time during which an adult gets infected has been worked out at 2 years 1 month. Too few adults contacts in this study have been followed-up for so long a period. Yet, it may be noted that whereas none, out of the 16 treated adult contacts followed up for 6 months to 3 years, became infected, in a comparable control group observed for a similar period. Out of 24, 6 became infected and one developed persistent lesions—early neural.

2. *Positive contacts (infected without lesions).*—Out of 36 treated positive contacts, none developed lesions, and 15 became negative for bacilli. There was no change in the remainder. Out of 43 positive contacts in the control group, one became a leprosy case (NT 1), 4 developed primary lesions, 7 became negative for bacilli and 31 remained static.

The average period during which positive contact develops primary lesions is 1 year 6 months in adults and 1 year 4 months in children. Only 10 positive contacts under prophylaxis have been followed up for periods over 1½ years. None developed any lesions. In the control group out of 17, 4 developed primary lesions and one became a leprosy case within 1½ years.

Out of the 15 treated positive contacts who became negative for bacilli, 1 (out of 5) became negative in 6 months, 10 (out of 21) in 1 year, 4 (out of 10) in 2 years. Of the 7 positive contacts in the control group who also became negative, 2 (out of 24) did so in 1 year, 3 (out of 9) in 2 years and 2 (out of 4) in three years.

Thus, among positive contacts observed during a period of 1 to 3 years :—

- (i) Out of 31 on prophylaxis, none developed lesions, 17 remained infected and 14 (45 per cent) became negative.

- (ii) Out of 37 on control, 1 became a leprosy case, 3 developed primary lesions, 26 remained infected and 7 (19 per cent) became negative.

3. *Contacts with primary lesions (macules without sensory impairment).*—In the treated group, out of 15 contacts with primary lesions, 2 became leprosy cases (NS2), 6 remained static, 3 remained infected but in these the lesions vanished, and 4 (26.5 per cent) became negative for bacilli and the lesions also vanished.

In the control group, out of 19 contacts with primary lesions, 2 became leprosy cases, 1 developed persistent lesion-early neural, 9 remained static, 5 remained infected but in these the lesions vanished, and 2 (10 per cent) became negative for bacilli and the lesions also vanished.

The above contacts had been observed for a period of 1 to 3 years, and it has been estimated that the time taken for a primary lesion to develop sensory impairment varies from 5 months to 3 years.

4. *Contacts with persistent lesions-early neural (Macules with sensory impairment).*—Two adult contacts with persistent lesions-early neural were put on prophylaxis for a period of 1 year to 2 years 8 months. One (observed for a period of 2 years 8 months) was rendered bacteriologically negative but the lesion persisted. There was no change in the other for a period of 1 year.

Of the 3 adult contacts with persistent lesion-early neural in the control group, 2 progressed into NT 1 stage in 1 year to 2 years 8 months. There was no change in the third observed for a period of 1 year.

From the results presented above a definite trend appears :—

- (i) DDS appears to be effective in preventing the infection of the uninfected contacts, and the development of lesions in the infected contacts, over an average period of 1 to 2 years.
- (ii) DDS in the doses used also appears to be fairly effective in rendering the host free of bacilli, its effect, however, decreasing with the progress that bacillary invasion had already attained before the prophylactic treatment was started. In contacts with primary lesions, DDS in the doses used cannot always be relied upon to prevent the progression of these primary lesions into anæsthetic macules. A higher dosage schedule in these cases seems indicated.

(B) *Field study (without detailed laboratory investigations) :*

119 lepromatous and 60 neural households are at present under observations. The total number of contacts in the lepromatous households in 448 and in the neural households is 411. Out of the above 448, 193 are under prophylaxis, and 255 are observed as controls. Of the 411, 159 are under prophylaxis, and 252 are controls.

These contacts have been on treatment or controlled observation, as the case may be, for a period of 1 year 6 months. So far none has developed definite lesions. As in the absence of bacteriological and immunological criteria the assessment of these results has to be made on the basis of the development of definite lesions in one or the other group, no conclusions can be reached at this stage.

IV. COMBINED TREATMENT WITH DDS AND INH.

(A) 533 lepromatous received combined treatment with DDS and INH in equal proportion for 6 to 24 months. Of these ;

1. (a) 7 had reaction and 1 had anæmia at the commencement of treatment ;
- (b) 82 got reaction, 28 developed anæmia and 26 developed both anæmia and reaction during the course of treatment.
2. (a) 81 improved bacteriologically; and
- (b) 24 were rendered negative for bacilli.

250 lepromatous cases received treatment with DDS alone for 6 to 24 months, and were observed as controls. Of these :

1. (a) One had anæmia and 9 had reaction at the commencement of treatment;
- (b) 21 developed anæmia, 47 got reaction and 11 developed both anæmia and reaction, during the course of treatment.
2. (a) 64 improved bacteriologically, and
- (b) four were rendered for bacilli.

V. B.C.G. VACCINATION IN LEPROSY

For the purposes of this evaluation, only those contacts who are tuberculin negative and negative or weakly positive to lepromin are considered eligible for further study and observation. Of those eligible, only every alternate contact is being vaccinated. The rest being observed as controls.

So far 33 contacts have been vaccinated this year.

VI. COMPARISON OF TUBERCULIN AND LEPROMIN REACTION IN CONTACTS.

(A) A comparative study in 195 contacts has shown a higher percentage (10 to 15 per cent higher) of lepromin positive reactors among the tuberculin positive contacts as compared to the percentage of lepromin positive reactors among the tuberculin negative contacts. However, a considerable percentage (varying from 58.3 in children from 0 to 6 years of age to 21.4 in adults 25 years and above) of tuberculin positive contacts remained negative to lepromin.

Whatever may be the explanation for the above results, it appears that at least among contacts of leprosy patients, the tuberculosis factor, plays a comparatively minor role, if at all, in the 'positivization' of the lepromin reaction and in the 0 to 6 years age group, it can be safely ignored.

(B) Out of 10 BCG vaccinated contacts who were retested 3 to 5 months later, only 5 became lepromin positive. Of these 5, 3 were originally lepromin positive 3 to 4 years before BCG vaccination, but had since become negative prior to it, and one was bacteriologically positive at the time BCG vaccination was carried out. The fifth could not be tested further. Surprisingly, none of the 8 (from the 10 above) tested showed any marked conversion in the tuberculin reaction.

VII. DESENSITIZATION IN LEPROSY

An attempt was made to desensitize 4 neural patients to lepromin by repeated injections of progressively increasing doses of lepromin (Dharmendra type). Lowering of the lepromin reaction (early as well as late) was observed in all the 4 cases.

VIII. THE VALUE OF ENZYME DIGESTION OF SKIN BIOPSY

Improvements in the methods of detection of lepra bacilli has been attempted through the use of pancreatic extract for digestion of the skin biopsy. For the purpose of assessment, the specimens already subjected to the chloroform-extraction method, were digested with the pancreatic extract at 37°C. for 24 to 48 hours and then examined for bacilli. Out of 81 biopsies so studied, in 22 cases both the methods proved negative; in 25 cases both were positive, but a greater number of bacilli were found after digestion; and in the remaining 34 cases the chloroform extraction method was negative, but this method of digestion showed a few bacilli.

IX. BLOOD GROUPS AND LEPROSY

A study of blood groups in 127 patients of different types has given the following analysis :—

Twenty-six belonged to group A, 47 to group B, 11 to group AB and 43 to group O.

91. Study of etiology and treatment of ulcers of foot in leprosy under Dr. B.B. Gokhale at the B.J. Medical College, Poona.

The following clinical methods have been studied to find out whether they can be applied to the assessment of the role played by the circulation in the aetiology of the ulcers on foot.

- (1) Oscillometer readings.
- (2) Histamine test.
- (3) Reflex Vase-dilatation test and skin-temperature.

The state of peripheral circulation was estimated in the following groups of cases :—

(1) Normal

(2) Leprosy cases where the disease is arrested and of normal type, with no deformity of finger or with deformity of finger but no ulceration.

(3) Leprosy cases with single or more trophic ulcers.

(4) Cases suffering from peripheral vascular diseases such as thrombo-angitis obliterans, Reynauld's disease, Scalene syndrome etc.

OSCILLOMETRY :

The oscillometer is an instrument which is capable of detecting, minute changes in the volume of the limb as created by the rhythmic introduction of blood into the extremities with each cardiac systole, and thus reflects the amount of blood flowing through the limb.

Low oscillometric index is thus indicative of reduced blood flow in a limb but by this method one cannot elucidate whether this is due to organic narrowing of the arteries or to vaso-spasm.

By this method six normal cases and fifty-one abnormal cases were studied.

NORMAL :

Six cases have been studied. Age in normal cases varied from 21-40 years.

In the normal cases, the systolic blood pressure varied from 124 to 90 mm. Hg and the diastolic blood pressure varied from 80 to 60 mm. Hg.

Difference in the systolic blood-pressure in superior and inferior extremities, is 30 mm. Hg. (The difference noted in U.S.A. is approximately 50 mm. Hg.) The blood pressure on both sides has been found to be identical.

The highest oscillometric readings (8.5) are recorded at mid-arm and at mid-thigh-level stand the lowest readings. (Traces to $\frac{1}{2}$) have been recorded across the hand and over the feet.

The average 0.1 noted in the normal cases.

Lower Extremity			Upper Extremity.		
Level	0.1 (average)	Range	Level	0.1 (average)	Range
Mid -thigh	6	4 —8.5	Mid-arm	5	2 —8.5
Below knee	7	3 —8	Fore-arm	4.5	1.8—7.5
Mid-leg	4.7	3 —6	Above wrist	4	1.5—8.5
Above ankle	4.5	1.5—6	Across hand	Traces to $\frac{1}{2}$	Traces to $\frac{1}{2}$

Oscillometric index on both sides is identical in normals.

FIRST GROUP :

This consists of Leprosy cases where the disease is arrested and of neural type, with no deformity of finger or with deformity of finger but no ulceration.

Twelve cases were studied.

Age varied from 16 years to 32 years. The systolic blood pressure varied from 120 mm. to 90 mm. Hg. and the diastolic varied from 70 mm. to 60 mm. Hg.

In four cases, the oscillometer readings were normal. In eight cases, the readings were below normal.

SECOND GROUP :

This consists of Leprosy cases with trophic ulcer or ulcers.

Seventeen cases were studied.

Age varied from 19 years to 55 years. The systolic blood pressure varied from 130 mm. to 90 mm. Hg. and the diastolic varied from 90 mm. to 50 mm. Hg.

The ulcer was present on one side in fifteen cases and on both sides in two cases.

In seven cases, the oscillometer readings were normal. In ten cases, the oscillometer readings were below normal. In four cases, out of these, readings were below normal on ulcer side, in one case below normal on non-ulcer side and in five cases below normal on both sides.

THIRD GROUP :

This consists of cases suffering from peripheral vascular diseases such as Thrombo-angitis Obliterans, Reynauld's disease, Scalene Syndrome etc.

Twenty-two cases are studied. The age varied from 12 years. to 55 years. The systolic blood pressure varied from 200 mm. to 90 mm. and the diastolic varied from 50 mm. to 110 mm. Hg.

In six cases, the readings were normal. In sixteen cases the readings were below normal on the affected side or sides. In one case, patient aged 45 years : systolic pressure was 200 mm. and diastolic pressure was 110 mm. Hg. This is a case of hypertension. In others the systolic pressure noticed from 130 mm. to 90 mm. Hg. and the diastolic pressure noticed from 90 to 50 mm. Hg.

HISTAMINE TEST :

This test gives an indication of Capillary Circulation, by the development of a wheal. It also evaluates the functional condition of nerves responsible for the flare. Some difficulty is experienced in the quantitative valuation of the flare due to the dark complexion of our patients.

By this method five normal and thirty-eight abnormal cases were studied.

NORMAL :

In normal cases the reaction started at 2.5 minutes and completed by 10-15 minutes.

FIRST GROUP :

This consists of Leprosy cases where the disease is arrested and is of neural type, with no deformity of finger or with deformity of finger but no ulceration.

Twelve cases are studied. In six cases, the response is normal and in six cases, the response is delayed.

SECOND GROUP :

This consists of leprosy cases with trophic ulcer or ulcers.

Twelve cases are studied.

In three cases the response recorded is normal. In nine cases, the response is delayed.

THIRD GROUP :

This consists of cases suffering from peripheral vascular diseases such as thromboangitis obliterans, scalene syndrome, Reynauld's disease etc.

Fourteen cases were studied. In five cases, normal response is recorded while in nine cases the response is delayed on the affected side.

REFLEX VASODILATATION TEST : (Foot immersion test)

The mechanism of this reflex Vasodilatation is not yet well understood but probably the warm blood returning from the heated limb forms the afferent pathway and sympathetic fibres to limbs form the efferent pathway of the reflex arc. This indicates the degree of the vaso-spasm, the affection of the vasomotor fibres and the occlusiveness of the vessels.

The degree of vasodilatation is determined by recording the changes of temperature in the digits. By this method three normal and five abnormal cases were studied.

In normal cases reflex vasodilatation started after 5 minutes. The rise of temperature is from 1-2°C. At the end of 30 minutes, the local rise of temperature of the immersed portion is 4.5°C.

FIRST GROUP :

Macule-anæsthetic type of leprosy cases. Three cases are studied. In these cases, reflex vasodilatation started after five minutes and completed by twenty minutes. The rise of temperature is 2.4°C. The local rise of temperature is 5-6°C.

SECOND GROUP :

Macule-anæsthetic type of leprosy cases with trophic ulcer on left side. The unaffected leg was immersed in water-bath.

Reflex vaso-dilatation started at 5 minutes and completed by twenty minutes. The rise of temperature was 1.14°C . The local rise of temperature was only 2°C .

THIRD GROUP :

Polyneuritic type of leprosy case.

Reflex vasodilatation started at five minutes and completed by twenty minutes. The rise of temperature was $0.6-1^{\circ}\text{C}$.

92. Metabolic studies on human leprosy under Dr. B.M. Braganca at the Indian Cancer Research Centre, Bombay.

During the current year, investigations on 'Metabolic studies on human leprosy' have mainly related to the respiratory metabolism of the organisms separated from lepromatous nodules. In the first stage of this work, attempts were made to separate the organisms from the lepromatous nodules, in a form which would be expected to retain the enzyme systems in an active state. It was found that the differential centrifugation procedures similar to those which have been widely used for separating different cellular components were effective for the purpose. The final fraction containing the organisms, was reasonably pure and contained only traces of tissue material as detected by the usual staining methods. Studies on the oxidative metabolism of the organisms have been started on fraction (L) separated from the lepromatous nodule. For this purpose, the fresh nodules obtained from the Achworth Leprosy home were kept frozen until sufficient number was collected for one respiration experiment. The oxidation was determined by the conventional Warburg manometric technique.

Results of several experiments show consistent oxygen uptake by the organisms both without the addition of any substrate and in presence of a heated extract prepared from nodular tissue. Although in some experiments higher oxygen uptake has been obtained with the addition of the heated extract, it is not possible to state with certainty at the moment whether the heated extract contains factors which significantly increase the oxidation over the endogenous metabolism. The serious difficulty experienced is due to the fact that the suspensions of organisms are very dilute and the total oxygen taken up by the material is not very considerable. Even though the nature of the substrate utilized by the organisms is not known, the results obtained so far indicate that the bulk of the organisms isolated from the tissue are able to respire *in vitro*. This suggests that the procedures used for separating the organisms from the nodule have not destroyed the enzyme systems of the bacteria and may be suitable for metabolic experiments. Since it is well known that the biological systems concerned with over-all oxygen uptake are very complex and involve several enzyme systems, it was of interest to

investigate the occurrence and activity of individual enzymes generally known to be concerned in oxidation processes. A beginning has been made in this direction by a study of the cytochrome oxidase. The results obtained with a few preparations indicate that this system occurs in the acid fast organisms separated from the lepromatous nodule. The activity of cytochrome oxidase was determined in the usual way by using paraphenylenediamine as the substrate. It appears from these findings that there is a lag period before the oxygen consumption proceeds more actively. Since it is known that the organism contains a lipoidal covering the lag may be explained by the inadequate and slow penetration of the substrate.

93. Leprosy research work under Dr. N. Mukerjee at the School of Tropical Medicine, Calcutta.

RESEARCH ACTIVITIES

I. HISTOLOGICAL STUDIES

The study of the flat hypopigmented macular lesions of leprosy with particular reference to the 'indeterminate' ones.—Biopsy specimen from 9 cases of leprosy with such lesions has been examined so far. On clinical grounds, 3 were classified as maculo-anæsthetic, 4 as 'indeterminate' and 2 shared the clinical characters of both. Six were slightly positive bacteriologically by the routine method and 3 were negative, the latter comprising one maculo-anæsthetic case, 1 'indeterminate' and 1 'doubtful'. Lepromin test was done in 8 cases, 6 giving negative reaction. A weak positive and doubtful reactions were found in 2 of the maculo-anæsthetic cases.

From the distribution and nature of the cellular infiltrate, the degree of affection of the dermal nerve twigs and the number of bacilli inside them, histologically, 3 of the above cases could be placed in between tuberculoid and leproma, and an equal number of cases could be placed towards the tuberculoid and lepromatous side of the classification-spectrum.

II. *Experimental transmission.*—Laboratory bred young Syrian hamsters and hybrid black mice have been utilized for this purpose. Preliminary investigation as to the susceptibility of these animals to Stephansky's bacillus indicated that the hybrid black mice were more susceptible to these organisms than white rats, the infection developing within 3 weeks, becoming generalized within 6 to 8 weeks, and the animals usually died $2\frac{1}{2}$ to 3 months after inoculation.

Attempt at experimental transmission of human leprosy to these animals has also been made. Some encouraging results have so far been obtained only in the Syrian hamsters. The intraperitoneal route of inoculation has been utilized for the present.

III. THERAPEUTIC STUDIES

(i) *Study of the effect of intracutaneous injection on leprosy lesions of acidomycin (Ogata) (Takada Pharmaceutical Industries, Japan):* Two

cases of the tuberculoid type, one of which was in the phase of reaction, have been treated so far. Both the cases were bacteriologically negative and lepromin positive. Infiltration of lesions with acidomycin was done on alternate days injecting about 0.05 c.c. at each spot. The daily dose was 0.25 c.c. gradually raised to 1 c.c. (=50 mg.). The total period of treatment was 9 months; and the average total dose of the drug injected was 3,270 mg.

No untoward effects were noticed. On the other hand, the injected lesions subsided completely, becoming flat and hyperpigmented. However, sensory recovery in the patches was not observed and nerve-thickening subsided to a slight extent only.

(ii) *Sandosten calcium* (Sandoz) and *Irgapyrin* (Geigy) in the treatment of reaction.—Sandosten calcium was given a limited trial for the treatment of reaction in 5 cases of leprosy (T-3; B-2). The drug was given intravenously in doses of 5 c.c. to 10 c.c. twice a week. Moderate improvement was found in 3 cases (T-1; B-2) after 2 to 3 week's treatment. From the results of this limited trial it is concluded that the drug has some effect in controlling reaction, but the beneficial effect was not found to be equal or superior to calcium—P.A.T.

Irgapyrin was tried for reaction with burning sensation all over the body and joint-pains in 2 cases of leprosy (L-1; T-1). The drug was given in doses of 5 c.c. intragluteally, 9 to 10 injections being given over a period of 18/27 days. However, owing to occasional febrile reaction after injection, the drug was subsequently given orally, one tablet thrice daily, over a period of 15/36 days. Further improvement followed. From the results of this limited trial, it can be concluded that Irgapyrin has some effect in controlling reaction and in this respect the oral method of administration is preferable than the parenteral one.

(iii) The value 'chlorpromazine hydrochloride' (Largactil, May & Baker) in relieving the pain due to leprous neuritis.—This was tried unsuccessfully for relieving the joint-pains and disturbed sleep developing in a lepromatous case, suffering from a chronic reaction persisting for about a year.

M A L A R I A

94. Insecticide and mosquito repellent inquiry under the Director Malaria Institute of India, Delhi.

During the period under review special attention was given to the problem of resistance of mosquitoes to chlorinated hydrocarbons and effectiveness of measures to control such resistant strains. Development of resistance to DDT in *C. fatigans*, *A. stephensi* and *A. fluviatilis* by exposing the adults to DDT deposits at each successive generation has progressed through many generations. Both the adult and larvæ of *C. fatigans* have shown considerable degree of tolerance to DDT. Studies

on *A. stephensi* have indicated that selection of both larvæ and adults hastened the development of resistance as compared to selection of either larvæ or adults only. *A. fluviatilis* has not developed any significant degree of resistance so far. The problem of resistance in *C. fatigans* was investigated from a biochemical standpoint. The average citric-acid content of the resistant strain was more than those of the susceptible strain.

Malathion and diazinon, two of the insecticides of phosphate series were effective for about 4 weeks against DDT resistant strain of *A. subpictus* and two weeks against DDT resistant *C. fatigans* when applied at the rate of 25 mg./sq. ft.

Comparative efficacy of 50 per cent dieldrin water-dispersible powder and 50 per cent dieldrin resin-water dispersible powder against mosquitoes and flies was studied. Dieldrin-resin formulations not only produced a high kill but the toxicity also lasted for longer periods when applied to mud panels in the laboratory.

It appears the sorption of dieldrin particles into the mud surfaces is retarded to some extent by the incorporation of resins with consequent increase in its biological activity. However, under field investigations incorporation of resins to dieldrin did not seem to prevent its sorption.

Efficacy of aerosol dispensers received from the World Health Organisation and a number of samples of mosquito control oils were tested and report submitted.

95. Inquiry on the preparation of water dispersible DDT under Dr. A.B. Biswas at the National Chemical Laboratory, Poona.

(A) *Micronization of DDT by spray atomization.*—With a view to prepare water-dispersible DDT powder, work on micronizing DDT by spray-atomization of the melt was undertaken as a first step. Using Nero's spray-drying unit (rotor speed ~30,000 r.p.m.), it was concluded that higher rotor speeds and larger size chamber were needed to obtain finer droplet size and to allow the supercooled droplets to crystallize out completely within the chamber. A completely different high speed (rotor speed ~2,000 r.p.s. or 120,000 r.p.m., 'Spinning-top' spray atomizer unit was, therefore, designed and fabricated. The salient features of this unit are :

- (a) A high speed rotor suspended while spinning on air-jet cushion over the stator thus virtually eliminating the high-speed bearing and lubrication problems.
- (b) Heating of the compressed air (driving the rotor) so as to heat up the rotor and maintain the desired chamber air temperature to effect optimum velocity of crystallization of DDT droplets.
- (c) A large size air-tight chamber.
- (d) Suitable collecting and suction arrangement.

(e) Controlled feed of the molten charge. The fabrication is completed and trials are now started.

The spray atomized droplets supercool and crystallize at a very slow rate. In order to determine the optimum conditions of crystallization, the velocity of crystallization of binary and ternary systems of technical DDT, as also of technical DDT after one and multi-stage purification were determined over the significant range of temperatures. It was found that the optimum velocity increases by about 4 and 13 times on one and two-stage purifications, respectively.

More than twenty potential nucleating agents were tested for increasing the rate of nucleation of supercooled DDT melts both under strictly controlled laboratory conditions as also by jet atomization.

(B) *Water dispersible DDT paste formulations.*—As an alternative to water-dispersible powders, DDT paste formulations, as reported earlier, have already been developed at the National Chemical Laboratory. The Public Health Department, Government of Bombay, being interested in the large scale utilization of these pastes, one ton of these formulations are currently being prepared for field trials in collaboration with the Public Health Department, Government of Bombay and the Poona Municipal Corporation.

96. Inquiry on the factors influencing the development of Malaria Parasite in Mosquito by Cytochemical Technique under Dr. H.N. Ray at the School Tropical Medicine, Calcutta.

(A) *Detection of fat during sporogonous cycle of Plasmodium gallinaceum in Aedes aegypti.*—Oökinete collected at the end of 24 hours after the infected blood meal showed a very faint Sudanophilic area covering almost the whole of cytoplasm. Nuclear area showed no reaction. By the 48th hour oöcysts were formed. In the oöcysts of this age, irregularly scattered Sudanophilic dots and rods could be seen. It was noticed that as the age of the oöcysts advanced the size and the intensity of the Sudanophilic bodies also increased appreciably. At 144th hour, when oöcysts had attained maturity the size of the Sudanophilic bodies had attained much larger dimensions and the staining reaction was markedly intense as compared with the previous stages. Accumulation of Sudanophilic substances in this sporozoa appeared to keep pace with age of the growing parasite and perhaps helped in the production of energy by their metabolism when the oöcysts finally liberated the sporozoites.

(B) *Effect of blood feed on the sporogonous cycle of Plasmodium gallinaceum in Aedes aegypti.*—Blood meal given at an interval of 48 and 72 hours after infective feed had some nutritive material which enhanced the rate of growth of oöcysts as compared to those from the batch fed on glucose solution only. It was also found that there was a rapid increase in the rate of growth of the oöcysts between 120th and 144th hour after the infective feed irrespective of post infective diet under prevailing conditions of temperature and humidity.

(C) *Effect of ookinete penetration in the stomach wall of Aedes aegypti.*—Both morphological and cytochemical pattern were changed in the host cell of stomach epithelium. Nucleus of the host cell became pressed out to one pole of the cell contour. In no instance, however, the pyroninophilic nucleolus, as was visible in the nuclei of normal stomach epithelial cells, could be detected in the nucleus of the affected cells. A prominent vacuole seemed to take the place of nucleolus in such cells. The phenomenon of vacuolization and loss of pyroninophilia in affected cells indicated the absence of ribonucleo-protein synthesis and the host were on the verge of degeneration.

(D) *Orientation of oöcysts of Plasmodium gallinaceum in the stomach wall of Aedes aegypti.*—The usual belief is that the ookinete pierced the stomach wall by forcing two of the stomach cells apart and ultimately came to lie under the outer envelope of the stomach (*Tunica elastica muscularis*, Hewitt, R. 1940, *Bird Malaria: The John Hopkins Press, Baltimore*). In course of our studies on the sporogony cycle of *P. gallinaceum* it was found that some oöcysts were intracellular in nature and caused cells damage to the host cell in gut epithelium of *Aedes aegypti*.

97. Inquiry on biochemical investigations in malaria with particular reference to protein metabolism of *P. gallinaceum* in chick malaria under Dr. M. Sirsi at the Indian Institute of Science, Bangalore.

The effect of anti-metabolites on experimental avian malaria (*P. gallinaceum* in chicks) and the study on the effect of several amino-acid antagonists and purine and vitamin analogues has been made on the course of infection in chicks infected with *P. gallinaceum*. Amino acids serve as building blocks in the formation of parasite proteins and other enzymes which act as co-factors and are intimately concerned with the functions of the protoplasm. Any effective anti-metabolite which interferes with this process of protein synthesis is likely to interfere with the normal growth and multiplication of the malarial parasite inside the red cell of the host. Also, the amino acid incorporation into protein is linked with the role of nucleic acids as templates for protein synthesis. A study of these analogues of amino acids and purines was thought to provide some information on the nutritional requirements of the malarial parasite. A study of these antagonists might also serve as models for the synthesis of potential chemotherapeutic agents in the treatment of malaria.

Effect of some antimetabolites in avian malaria.—Of the twelve anti-metabolites studied, gluco-ascorbic acid, pyridine-3-sulphonic acid desthiobiotin, neopyrithiamine, taurine, pantoyl taurine were vitamin antagonists., DL-ethionine, allyl glycine, 6-methyl tryptophan were amino acid antagonists, while thiouracil, benzimidazole and 2-amino-4-methyl pyrimidine belonged to purine antagonists group.

Since these anti-metabolites might affect adversely the host also, the histological study of the tissues of the birds treated with these drugs were also made. The results of the studies show that 6-methyl tryptophan and

DL-ethionine exhibit suppressive action on the malarial parasite without any tissue damage to the liver or kidneys. Allyl-glycine and methionine sulphoxide have been found to be toxic to the animals. Among the purine analogues, benzimidazole and 2-amino-4 methyl pyrimidine have given encouraging results by prolonging the incubation period and survival rate. The anti-vitamins desthiobiotin, neopyrithiamine and pyridine-3-sulphonic acid have shown some suppressive activity, whereas pantoyl taurine, gluco-ascorbic acid exhibited toxicity to the host.

98. Inquiry on the mechanism of the maintenance of plague infection in rats and fleas under Dr. S.C. Seal at the All-India Institute of Hygiene & Public Health, Calcutta.

The scope and purpose of the study.—One of the questions that needed an answer in regard to the re-appearance of plague in certain places of endemic zones at long intervals is whether it is always or often due to the result of importation or of recrudescence of the simmering or the dormant subterranean local infection. However, during the investigational work at Calcutta evidence of existence of chronic infection of plague in the rat population was obtained.

The purpose of the present investigation was to find out the mechanism of persistence of infection among the commensal rats and fleas which would lead to the periodic recrudescence of plague infection without importation. Involved in this study are the question of persistence of infection in fleas, existence of chronic plague in domestic and wild rodents, change in virulence of plague organism, immunity in rats in different seasons, transference of infection through eating of carcasses of infected rats, etc.

Methods of study.—These have already been described in previous reports. A large-scale breeding of pure strain fleas has also been arranged in the Laboratory. The various possibilities kept in view were : (i) Bacteriæmia as a sort of relapse followed by recovery, (ii) the relapse followed by acute plague and death, and (iii) continued survival with or without any evidence of chronic infection.

The purposes of experiments with immunized rats were : (i) To find out whether chronic foci arise by infecting them naturally or artificially, (ii) whether some of them could infect the susceptible rats through fleas, (iii) the difference in behaviour between the immunity acquired by surviving from natural infection and that by artificial methods, and (iv) whether the immunity thus acquired fluctuates according to seasons.

For the purpose of safe transmission experiment a convenient device has been made consisting of a large glass or wooden chamber to accommodate several closed experimental glass boxes with single or double wire cages. A large population of pure-bred fleas especially *X. cheopis* has been maintained and the supply of commensal rats is obtained through the courtesy of the West Bengal Plague Control Laboratory at Calcutta.

Summary of the results.—Altogether 17 experiments have been conducted. These indicated that the organisms soon became deteriorated as the epidemic proceeded and the infection was maintained for a prolonged period in those infected animals which survived the epidemic and that it was possible for the fleas (*X. cheopis*) to imbibe the infection from such animals when the latter was under the spell of bacteræmic relapses, and to transmit it to other susceptible hosts. In regard to the seasonal variation, in the experiments started in May, there was prolongation of the incubation period up to January. In February and March the interval became gradually shortened, as generally noted during the epidemic phase, and it became prolonged again from the end of March. Three factors are likely to be involved in this observation, viz., variation of the resistance of the rats, increase in virulence of the organism and the ecology of fleas and rats. Series of experiments carried out on the above lines have so far yielded results in favour of these multiple factors being involved.

Subsequent experiments have yielded the following results :

- (i) When the infection is being maintained in the immunized rats, introduction of non-immunized rats leads to reduction of the incubation period and gain in virulence of the organism.
- (ii) The protein-immunized rats give evidence of greater resistance than the Hoffkine vaccine immunized rats.
- (iii) Even though the virulence of the organism was reduced in the immunized rats infection could still be transferred to non-immunized rats by the fleas.
- (iv) A surviving rat when killed and examined on the 83rd day was found positive of non-virulent *P. pestis*.
- (v) The average cage age life of *R. rattus* without infection was 36 days and of *B. bengalensis* 33 days.

99. Inquiry on the control of *W. Mialayi* Filariasis in Kerala State under the Director, Malaria Institute of India, Delhi.

Objects of the Studies.—To test the efficacy of diethylcarbamazine in the control of *W. malayi* filariasis.

Work carried out during the year by the unit at Shertallai, Kerala State.

(a) Follow up studies for a year were concluded in seven batches of volunteers placed on different dosage schedules during the previous year and also in six other groups started during the current year.

(b) Mass therapy in a dose of about 4 mg./kg. of diethylcarbamazine in a population of nearly 10,000 was carried out. Additional studies were also carried out.

RESULTS

(a) Follow-up studies in persons put on the following dose schedules.

- (i) 4 mg./kg. of diethylcarbamazine for five days, alone and combined with 25 mg. vit. C or 10 gr. of A.P.C.
- (ii) 5 mg./kg. of diethylcarbamazine for four days alone and combined with histantine or cal-gluconate.
- (iii) 6 mg./kg. diethylcarbamazine alone for three days.
- (iv) 4 mg./kg. diethylcarbamazine once a week for 2 and 4 weeks.

In all these cases the microfilarial counts were done on the day prior to and following completion of treatment and 25, 75 and 110 days later (except in *group iv*). Routine blood and stool examination was also done. Monthly inquiries regarding the "clinical state" of the treated cases were also instituted.

Results obtained.—There was a marked drop in the microfilarial counts following the therapy in all the groups. This effect was observed to continue in most cases till the end of the observation period of one year. The few cases which showed an increase never reached the pre-therapy level.

2. *Reactions.*—One or more of the following types of reactions were noted during therapy, starting from 12 to 24 hours of initial dosage. Reactions consisted of headache, nausea, vomiting, body pains, bowel upsets and fever which reached the peak on 2nd day and came down by 5th or 6th day.

In the group receiving 4 mg./kg. weekly, the fever and other reactions were of shorter duration as compared to the other groups. The only reaction observed during the second week of therapy, was body pains and in the 3rd and 4th weeks of therapy no reactions were observed.

(b) *Mass therapy.*—Mass therapy with diethylcarbamazine was carried out during March/April, 1957, in Kadakkarapally area (Shertallai Taluk) with a population of about 10,000. The schedule consisted of a five-day course of treatment with a single dose of 200, 156, 100 and 50 mg. of dihydrogen-citrate salt to adults, adolescents, children between 6 and 12 years and between 2 and 6 years, respectively. The drug was distributed by locally trained people with the help of local volunteers. In most of the cases the actual consumption of the drug was ensured. The area was divided into six wards each with approximately 1,500 population.

Prior to starting of therapy, a night survey of 4,699 persons showed that the infection was entirely *Mf. malayi*. The infection, disease and endemicity rates were 17.66, 16.3 and 33.4 per cent, respectively.

The *comparison area* was Thannirmukam (Shertallai) with a population of about 5,000. Filarial survey showed that the prevalent infection

was *W. malayi*. The infection, disease and endemicity rates were 12.5, 8.0 and 20.13 per cent, respectively.

The results of therapy showed that 79.7 per cent of people took the drug. Of these 80.1 per cent took the full course, 7.85 for 4 days, 4.45 per cent for 3 days and 3 per cent for 2 days and 4.6 per cent for one day only.

Reaction was highest in adults (78.18 per cent). In younger age groups reactions were less though in a few cases high fever, convulsions, vomiting and abdominal colic were noted.

51.0 per cent of persons on therapy showed some reaction or other. In addition to other reactions, a few cases of urticaria were noted. A few cases of lymphadenitis turning to abscess were noted. A number of persons (about 20 per cent) of all age groups, passed round worms during therapy.

Minor reactions were treated by APC, sodamint tablets by the drug distributors. Those needing medical attention were treated at two or three centres where medical personnel were stationed.

Pre- and post-therapy mosquito collections and clinical inquiries about filarial attacks from selected houses are being carried out at regular intervals.

(c) *Collateral studies :*

- (i) *Microfilariae in peripheral blood during general anæsthesia.*—In animals even with light filarial infections induction of general anæsthesia has been reported to bring to the peripheral circulation large number of microfilariae. Observations on 14 residents of Shertallai, who were operated under general anæsthesia showed no microfilariae. Operations were done during the day time. 5 of these cases were positive for *Mf. malayi* on the previous night.
- (ii) *Congenital filariasis.*—8 of 50 women awaiting confinement were noted positive to *Mf. malayi*. After delivery placental, umbilical and infant blood were examined. No positives were noted in any of these specimens examined.

100. Inquiry on the epidemiology of filariasis in West Bengal under Dr. P. Sen at the School of Tropical Medicine, Calcutta.

As in last year localized representative areas of a few more districts, viz., Burdwan, Hooghly and Howrah on the West, and Jalpaiguri and Darjeeling on the north of the State surveyed and the results obtained are discussed below under the districts separately. In assessing the filarial disease rates only definite manifestations of elephantoid condition of limbs and scrotum or other organs were considered.

Burdwan.—A check survey of both urban and rural areas within the district was carried out during the period October to December, 1956.

Burdwan proper (ward No. 4) and Asansol (ward No. 4) were taken as representatives of urban area, and for rural area investigations were carried out in Galsi, Bhiringi and Birbhanpur (both near Durgapur), Ismail—a village near Asansol and Memari. Altogether 2,007 persons were examined involving different areas in the district of Burdwan; of these 131 showed definite symptoms of the disease giving the overall disease rate as 6·5 per cent. The filarial infection rate for the entire area comes to 8·7 per cent. *Culex fatigans* mosquitoes dissected in the district was 2,217; out of these 35 showed the infection giving the overall mosquito infection rate as 1·5 per cent. Infective stages of *fatigans* were recorded from Galsi and Durgapur areas.

Hooghly.—The following areas, both urban and rural, within the district were surveyed: Chandernagar and Kotrung as representatives of urban area and Pandua, Khalisani (near Chandernagar) and Arambagh as examples of rural area. Altogether 1,400 persons were examined in this district as represented by the above areas. Out of these, 29 showed definite manifestations of the disease and 74 were having the bancroftian infection in the blood 3, the overall disease rate was thus 2·1 per cent and filarial infection rate 5·3 per cent. *Culex fatigans* to the total of 1,341 was dissected of which 19 were positive for the filarial larvæ giving a composite infection rate in the mosquito as 1·4 per cent.

Howrah.—The jute industrial area of Bally as a representative of urban area in the district of Howrah and three rural areas under Uluberia, Amta and Andul were investigated. When all the areas investigated within the district are taken together, the disease rate comes to 2·4 per cent and the filarial infection rate to 2·2 per cent. We were able to examine 890 persons in the district, out of which 21 had manifestations of filariasis and 20 were positive for microfilariæ in blood. Mosquito infectivity was rare in the district excepting in urban areas as in Bally where also the rate was as low as 1·2 per cent, the species involved being *C. fatigans*.

Jalpaiguri.—In Jalpaiguri, ward No. 3 and part of ward 5 involving a total population of 3,998 were surveyed as a token of urban area, and persons actually examined were 417. Of these 6 showed elephantoid conditions and the disease rate thus was 1·4 per cent only. Number of persons with microfilarial infection of the bancroftian type in blood was 23 giving the filarial infection rate as 5·5 per cent. Total mosquitoes dissected in the town were 484 *C. fatigans* and 82 *A. vagus* and *A. subpictus* together, but none proved positive.

In the rural area, Moulvipara (union 8 Mandalghat) inhabited by Muslim community and Rakhaldebi (Union Kharia)—a Hindu predominating village within 4 to 5 miles of the town of Jalpaiguri were surveyed. In the Muslim village, strikingly enough, there was no evidence of the prevalence of the disease, while in the Hindu village 3 persons with filarial involvement were noticed giving the disease rate as 2·6 per cent. There were 7 persons in this village with filarial infection which gives the infection rate as 6·0 per cent.

Taking the sum total of the work done in the district, it was found that out of a total population of 4,828 resident in the areas 533 persons were examined of which 9 had filarial manifestations giving the disease rate as 1·7 per cent. Blood of 30 persons was positive for microfilariae of bancroftian type and this brings the filarial infection rate to 5·6 per cent. The vector concerned in the area which has very low incidence of the disease remained undetected, although *Culex fatigans* appears to be the species concerned in all probability.

Darjeeling.—Siliguri municipal wards Nos. 1 and 5, and Rangapani about 9 miles up Siliguri were investigated as representatives of urban and rural areas in the district. We were able to examine 508 persons out of a population of 5,021 in Siliguri, in the wards investigated. Of these only 9 persons had filarial manifestations giving the disease rate as 1·8 per cent. Blood of persons on examinations showed 19 positives for bancroftian microfilariae which give the filarial infection rate as 3·7 per cent. In the town *Culex fatigans* was heavily breeding in sullage water, and out of a total dissection of 346 mosquitoes 7 were positive giving the mosquito infection rate as 2·0 per cent.

In Rangapani out of a total population of 375, mostly indigenous people, we examined 77 persons ; of which one only had symptoms of the disease giving the rate as 1·3 per cent. Of the persons examined 7 showed the presence of microfilariae in the blood and the filarial infection rate was found to be 9·1 per cent.

When the district is considered as a whole from the observations so far carried out, the overall disease rate comes to 1·7 per cent out of an examination of 585 persons in the areas where the total population involved was 5,396. Altogether, 26 persons among the persons examined showed microfilariae in their blood giving an overall filarial infection rate of 4·4 per cent. *Culex fatigans* appeared to be the only vector concerned in the transmission in the district where the parasite concerned is *W. bancrofti*.

Other studies undertaken.—The eosinophilia in filarial subjects has been investigated in certain areas involving both bancroftian and malayi types of parasites. Majority of the persons over 54 per cent, showed eosinophilia of 1 to 10 per cent ; the next highest group about 30 per cent had eosinophilic count of 11 to 20 per cent in thin slides. The maximum eosinophil count was 40 per cent. The earliest age showing elephantiasis was in a child of 13 years with swelling in left leg.

Blood of persons positive for microfilariae has been collected at different hours, both during day and night, to study the periodicity of the filarial parasites. The data have accumulated and the result of the study will form the subject of another paper. We have met with the parasites in blood taken in the afternoon at about 5 p.m. but none so far among slides taken after 7 in the morning.

Several instances of cattle microfilariae have come to our notice, and these are from malayi area and it would be worth following up the

systematic position of the parasites and if there is animal reservoir of the human filariæ.

Analysis of digestive blood of the mosquitoes, particularly *A. hyrcanus* var *nigerrimus* have been carried out by precipitin tests and a large proportion of the digested blood proved to be of human source.

101. Studies on the general behaviour of vector species of anopheline under the Director, Malaria Institute of India, Delhi.

In 12 states, namely, Andhra, Assam, Bengal, Bihar, Bombay, Delhi, Madras, Mysore, Punjab, U.P., M.P. and Rajasthan, these investigations were carried out. During the period under review, top priority was given to the determination of susceptibility levels of mosquitoes to chlorinated hydrocarbons. *A. subpictus* in some of the sprayed villages in Delhi State is resistant to DDT. *A. subpictus* larvæ from the sprayed village also showed significantly lowered susceptibility to DDT than those from the unsprayed village.

A standard test method for determining the susceptibility of adult culicine mosquitoes is being developed as the Busvine-Nash method for measuring the susceptibility of anopheline mosquitoes is not applicable to culicine mosquitoes. Due to high natural insecticidal tolerance of *C. fatigans* critical range of mortality was not obtained with one hour exposure to highest DDT concentration possible in white oil. Benzene white oil solution and aromex have been tried as solvents. Some other solvents for DDT are being investigated. 'Studies on the comparative efficacy against anopheline mosquitoes of DDT (100 mg./sq. ft.) diazinon (25 mg./sq. ft.) and malathion (25 mg./sq. ft.) water-dispersible powder were carried out in the villages of District Udaipur (Rajasthan) during July, 1957. This area had never been sprayed with any insecticide previously. From the entomological data collected, it appears that the mosquito density started building up in the sixth week in malathion and diazinon sprayed villages and in the 12th week after spray in the DDT treated villages.

102. Experimental study of the difference in response are the tissue injury in an animal suffering from malaria as compared to response in normal animals under Dr. K.C. Basu Mallick at the Nilratan Sircar Medical College, Calcutta.

Liver damage has been caused in albino rats by carbon tetrachloride, heat injury, cold injury, and partial hepatectomy in normal animals and in animals infected with *P. berghei* to find out if the plasmol dial infection increases the vulnerability of the organ to the injurious effects of these noxious agents. Nephrotoxic serum has been injected into normal rate and infected rats and the renal changes in these two groups of animals are being studied.

103. Ultrasonic methods for preparation of DDT Suspensions for use in malaria control under Dr. R.S. Krishnan at the Indian Institute of Science, Bangalore.

A high power ultrasonic generator suitable for studies of emulsification and dispersion has been constructed. A suitable reaction chamber for studies of the parameters connected with the emulsions has also been set up. A number of stable emulsions of liquids immiscible with water (e.g. benzene, kerosene) have been prepared and their stability tested. These methods are being directly applied to the preparation of DDT emulsions.

104. Study of the distribution, breeding habits and infectivity of culicoides, *Atrichopogon* and *Palpomyia* (*Ceratopogonidae*) under Dr. P. Sen at the School of Tropical Medicine, Calcutta.

The object of the present study is to seek clarification of the various aspects in the *Ceratopogonidae* mainly connected with the breeding habits, distribution and infectivity of the different genera. Particular attention was paid in working out the systematics of midges of the *Culicoides* genus. Our observations covering these aspects are detailed below.

DISTRIBUTION

1. *Study by light trap method.*—Altogether 3,221 specimens of *Culicoides* in addition to a large number of other genera mentioned in the title were collected by this means. The collection comprised of at least 15 distinct species of which 5 only were previously known. The light traps were placed in various places both in and around the city as well as in distant areas, such as Jhargram (Midnapore) and Bolpur (Birbhum). The 5 known species mentioned above are *C. peregrinus*, *C. anophelis*, *C. similis*, *C. clavipalpis* and *C. macfiei*. Other species recorded appear new to the science and these have been named and a key prepared.

Of the collections made the species *C. alatus* has the widest distribution being about 80 to 90 per cent of the total catch of the different midges in traps. The sexes in the species are represented in equal proportion. The insects are positively phototropic and the females caught in front of light showed different egg stages both mature and immature. The nature of blood feed was determined in some by precipitin tests.

2. *Study by sticky trap method.*—The sticky traps were prepared from waste x-ray films painted with 'Ostico' on one side. These were placed in open rolled up like chimney glass. In 65 days using 172 such traps 8 species of *Culicoides* including those mentioned above and altogether 1,628 specimens were collected by this means. The seasonal distribution of the various species is also being worked out.

Breeding habits.—Breeding of the various species recorded was noted from rotten banana vegetation, mud, algal growth, tree holes, edge of ponds and also from clean water. Of the five species collected in course of actual breeding the *alatus* and *similis* were from muddy soil while *paipifer* and the other two undescribed species were from rotten banana vegetation.

The larval stages of *alatus* and *palpifer* occupied 7 to 10 days, *peregrinus* also had almost similar larval life. The pupal stage lasted for 3 days in *palpifer* 5 days in *alatus* and 6 days in *peregrinus*. One *Atrichopogon* sp. took 28 days to complete its life cycle. The female of this species laid 35 eggs in one lot.

Infectivity.—In course of dissection of wild caught *alatus* and *peregrinus* we came across with certain *Mermis*-like worm in the abdomen of the males of the species. The helminth measured 2.7 mm. in length and 0.6 mm. in breadth.

Anatomical.—The alimentary canal of the insect *C. alatus* is almost a stright tube and begins at the wide pharynx with thick muscular lining. The common salivary duct opens near the base of the hypopharynx. The œsophagus is a narrow tube having a dorsal diverticulum for storage of food matter. The stomach in the species is wide and saccular and at its junction with the hindgut two malpighian tubes are situated. These are long structures and after a forward course turn downwards ending near rectum which is highly dilated and shows two papillæ on its surface.

The female reproductive system has the usual components. One very important structure of great taxonomic importance appears to be the spermathecae which may be 2 or 3 in number. The third is usually rudimentary. The male reproductive organs also show the usual components. The external genitalia and the structures of the hypopygium possess great taxonomic importance in identifying male *Culicoides*.

Systematics of Culicoides.—The systematics of the different species recorded from this country, including those collected by us, have been fully worked out and a revised key to include all known and new species has been made ready for publication. Of the 15 species 10 have been newly described or going to be described. Apart from wing pattern and ornamentation of dorsum the spermathecae and hypopygium have been utilized in sorting the different species.

The males of *C. anophelis* have been recorded for the first time by us. The species shows certain primitive characters which when followed in earnest might lead to a better understanding of the phylogeny of the group of midges.

TUBERCULOSIS

105. Tuberculosis Survey under Dr. P.V. Benjamin, Tuberculosis Adviser to the Government of India, Directorate-General of Health Services, New Delhi.

The tuberculosis survey in various zones was started under the auspices of the I.C.M.R. towards the end of 1955 and the beginning of 1956.

The findings of preliminary report were only tentative and may be summarized in brief as follows :—

(a) The morbidity varies from 7 to 30 per 1,000 population in the different areas.

(b) There is no marked difference as regards the prevalence of the disease in relation to the size of the population, i.e., accessible villages, small towns and cities.

(c) The morbidity rates are lower for females than for males and both show a continuous increase with age.

(d) The prevalence in the age group 35 years and over was considerably higher than in the age group 5 to 34 years ; and

(e) The rate of bacteriologically positive cases for 1,000 population has shown a variation from 1 to 11 in the different areas and zones.

The data used in the preliminary report suffer from many limitations which make comparisons between zones extremely difficult. The field work was planned to be completed by 31st March, 1957, but owing to unforeseen circumstances, it was not possible to stick to the schedule. The progress of work done up to the end of September, 1957 was reported to the TB Sub-Committee at Lucknow in December, 1957. Till then field work had been completed in Delhi, Hyderabad, Madanapalle and Trivandrum zones. In Patna zone two blocks in Patna City which were inaccessible to the mobile x-ray unit had remained to be covered. The Patna Survey Unit is now trying to complete work in these blocks also and it is hoped that both the blocks will be covered by the end of March, 1958. In Calcutta zone the work was stopped on 29th May, 1957 due to the breakdown of the x-ray machine. One of the two portable x-ray units supplied by the WHO was sent to Calcutta and the work was resumed on the 24th November, 1957. Up to September, 1957, the Calcutta Unit had covered 29 out of the 40 blocks in Calcutta City, 4 of the 6 towns and 3 of the 30 villages. It was decided at the Annual Meeting of the TB Sub-Committee that the unit should complete work in the remaining blocks in Calcutta City first with the portable x-ray unit.

In connection with the bacteriology tests a special study was undertaken under directions of the T.B. Sub-Committee by Mr. R.M. Barton, Clinical Pathologist of the U.M.T. Sanatorium and Dr. E. Mossige, the WHO Bacteriologist, who visited the laboratories of the Survey units and studied the techniques of bacteriological examinations, collection of sputum etc. adopted by them.

106. Scheme of Tuberculin Retesting of Mass. B.C.G. Campaign Vaccinated Persons in India under Dr. P.V. Benjamin, Tuberculosis Adviser, Directorate-General of Health Services, New Delhi.

The broad aims and objects of this inquiry are :—

1. To assess the degree of allergy imparted by mass campaign vaccination and variation, if any, from place to place.
2. To check on the potency of vaccine—batch to batch.
3. To check the technique of vaccination at different places.

The general assessment on these lines was started towards the middle of 1955. Since then, several preliminary reports have been submitted to the I.C.M.R.

The results of the general re-testing showed that there was a tendency for the post-vaccination allergy to wane out gradually after a period of two to three months of vaccination and boost up again after twenty months. A special study to investigate into the effect of time on post-vaccination allergy was taken up on the recommendations of the Tuberculosis Sub-Committee of the I.C.M.R. This work was started in September, 1957.

The assessment Team re-tested about 7,500 school children during the period November, 1956 to September, 1957 in 9 different States. The mean indurations for the groups varied from 9.6 mm. to 16.1 mm., most of the groups showing between 11 mm. and 14 mm.

The Team also carried out about 8,000 pre-vaccination testing in six different States.

To study the differences in the technique of tuberculin testing, reading and vaccination, the Assessment Team doctor and three technicians selected at random from the State Teams carried out pre-vaccination testing in four States. In most of the cases, differences were

observed both in the technique of testing and reading. Re-testing by the Assessment Team showed that the post-vaccination allergy in groups vaccinated by different 'technicians' differ significantly only in a few cases.

To study the effect of time on post-vaccination allergy about 6,000 school children each were tested simultaneously in four localities in Bombay State by four technicians selected at random. The non-reactors were vaccinated with four batches of vaccine. These groups will be re-tested at four intervals according to the 4×4 Latin Square design adopted for the study.

107. Inquiry into the virulence guineapigs and mice of tubercle bacilli isolated from tuberculous patients prior to, during and after treatment streptomycin, PAS and isoniazid, under Dr. J. Frimodt-Moller, at the Union Mission Tuberculosis Sanatorium, Arogyavaram P.O., Chittoor Distt.

During the year 37 strains isolated from patients were submitted to virulence tests; 30 of these tests were completed. Another 37 strains previously tested for virulence were retested. Seven strains from patients in the United Kingdom were also tested. This gives a total of 81 strains tested for virulence during the year.

Although the main purpose of the inquiry is to study possible changes in the virulence caused by the influence of drugs during treatment of tuberculous patients, especially after treatment with isoniazid, an unexpected observation has emerged as the work proceeded. In order to have a base line for estimating changes in virulence with the present technique, a number of strains from Indian patients with fresh untreated tuberculosis were examined. Whereas such strains according to the general experience in Western countries should be of a fairly uniform high virulence unless they were obtained from special types of tuberculosis such as lupus vulgaris, it became soon evident that strains obtained here from Indian patients with fresh pulmonary tuberculosis had a greater range of virulence as more than 50 per cent produced much less tuberculosis in the guineapigs than was expected.

While the work of examining strains from cases treated with drugs has continued, special attention was given during the year under review to finding out if the occurrence of strains with low or moderately low virulence could be explained by mere variations in the technique employed or whether it could be rightly assumed that Indian strains may be of low virulence. Three lines of approach were adopted : (i) to repeat the tests on a number of strains already tested, (ii) to compare the susceptibility of Indian guineapigs with that of European animals, and (iii) to examine tubercle bacilli obtained from European patients with fresh untreated tuberculosis with our own technique. The results so far have been as follows :

1. *Virulence tests repeated.*—Thirtyseven strains, (fresh, sensitive or resistant) were re-examined by being submitted to a second virulence

test in guineapigs. Each test consisted of the inoculation of 10^{+2} , 10^{+4} 10^{-6} milligram culture intraperitoneally into 2 guineapigs each. One animal of each pair were killed at 4 weeks, the others at 8 weeks. The results showed, as could be expected, some variation as compared with the original tests, but there was a clear positive correlation between the virulences of the first and the second tests. These findings suggest that the technique is consistent and the results reproducible.

2. *Susceptibility of guineapigs.*—In order to compare the susceptibility of our Indian guineapigs with European animals, twelve young white animals (10 females and 2 males) were imported from the Laboratory Animals Bureau of the Medical Research Council Laboratories, Hampstead, London. They belonged to the "K-strain" which is considered particularly susceptible to infection with tubercle bacilli. When a sufficient number of young animals had been bred in our laboratory the first experiment was done by inoculating on 12-3-1957, 11 British and 11 Indian guineapigs of same age and weights with 10^{-6} mg. culture of tubercle bacilli intraperitoneally of a strain with known high virulence. Of the Indian group the first animal died of generalized tuberculosis on the 76th day, the last on the 162nd day, the mean survival time being 115.8 days ($SE=9.04$). Of the British animals one died on the 23rd day of a non-tuberculous cause while the first animal to die from tuberculosis died on the 126th day. When all the eleven Indian animals had died, there were still 7 British alive, and at the 350th day after inoculation 3 of these were still alive. The mean survival time of the British animals on this day was 212.1 days ($SE=32.53$). The difference in mean survival time between the two groups is highly significant ($t=2.88$, $P=0.005-0.001$).

Another similar experiment was done on 27-6-1957 using ten animals of each breed and inoculating the same strain and dose as before. The Indian animals, showed a mean survival time of 83.3 days ($SE=9.66$), the first dying on the 46th, the last on the 125th day. Of the British animals the first died on the 71st day; on the 125th day when all the ten Indian animals had died only 4 British had died. On the 240th day one British animal was still alive. On this day the mean survival time was 157.5 days ($SE=18.98$). Again the difference in mean survival time is highly significant ($t=3.49$, $P=0.005-0.001$).

From this comparison it is obvious that our Indian stock animals are by no means less susceptible but rather more so than the British breed although this was thought to be a specially susceptible strain.

These findings support further the conclusion that the moderate or low virulence demonstrated among strains of tubercle bacilli isolated from the Indian patients with fresh untreated tuberculosis is a true observation and cannot be ascribed to an unusually high resistance among our Indian experimental animals.

3. *Virulence of strains from European patients.*—Through courtesy of Dr. D.A. Mitchison of the Tuberculosis Chemotherapy Centre, Madras, seven strains of tubercle bacilli isolated from British patients with untreated tuberculosis were obtained. After subculture they

were submitted to virulence tests in the same way as indicated above. All seven showed high virulence, the five even in a very high degree. Of 41 Indian strains from cases of untreated tuberculosis examined by us until now only 7 showed the same degree of virulence as the latter.

Although the sample of foreign strains is small, these findings again confirm our impression that European strains are usually of high virulence while our Indian strains may often be of lower virulence. The number of foreign strains examined by us is being increased.

4. *Conclusion.*—The present inquiry has led to the demonstration of an unusual variation in the virulence of tubercle bacilli isolated from Indian patients with fresh untreated tuberculosis. Evidence has been produced to show that demonstration of a high proportion of strains with low or moderately low guineapig virulence is real and not due to experimental errors.

The importance of these findings should not be underestimated as they may have a bearing upon other unusual observations in the field of tuberculosis, such as the wide-spread tuberculin sensitivity of unusually low degree, first demonstrated in Madanapalle and subsequently found elsewhere in India as well as in other countries in the tropical region. These findings may also be related to the observation that drug treatment often appears to lead to an unusually quick absorption of the lesions in pulmonary tuberculosis in Indian patients. The latter observation is not yet substantiated by facts and figures but our material should soon be sufficiently large to enable us to correlate the virulence of the tubercle bacilli to the type of disease in the patients from whom they have been isolated and to the response of the patients to drug therapy.

108. Inquiry on the incidence of human and bovine type of infection in extra pulmonary tuberculosis with or without associated pulmonary tuberculosis under Dr. N.L. Chitkara and Dr. M.L. Sur at the Medical College, Amritsar.

Eighty two cases were taken up for investigation.

Clinical diagnosis.—Out of a total of 82 cases, abdominal tuberculosis alone was present in 35 cases and associated tubercular lymphadenitis (cervical or axillary) in another 10 cases. Isolated tubercular lymphadenitis was diagnosed in 28 cases; 2 cases had cold abscess; 2 were diagnosed as polyserositis; 3 were diagnosed as tubercular meningitis; 1 as pleural effusion and 1 had tubercular emphysema.

Age and sex incidence.—The ages of the patients at the onset of their illness varied from 5 to 37 years. Out of 82 cases, 62 were females and 20 were males.

Duration of symptoms.—Before coming under observation, the patients suffered from symptoms of disease for a variable length of time ranging between 10 days to as much as 15 years.

Various investigations done.—Hæmoglobin estimation, total and differential leucocyte count, erythrocyte sedimentation rate, naked eye and microscopic examination of stools and complete examination of urine were the routine investigations done in all cases. Hb. level varied from 4.1 g. (2 cases below 6 g.) to 14 g. per cent with average of 10.24 g. per cent. ESR, figures varied from 3 mm. (3 cases below 10 mm.) to 125 mm (8 cases above 100 mm.) in the 1st hour by Westergren method.

Gastric lavage and sputum examination.—An attempt was made to examine sputum and or gastric lavage by smear and culture methods for A.F.B. in all cases for at least 3 times but it was not possible in every case.

Mantoux test was done in all cases up to 1 in 1,000 dilution of old tuberculin. It was positive in all except one which was excluded from the series on further investigations.

Radiological examination.—Skiagram of the chest was taken in 76 cases and radioscopic examination in another 3 cases. Forty-five cases showed no abnormality. Twelve showed enlargement of bronchial or mediastinal lymph nodes, 12 cases showed parenchymatous infiltration, 5 showed pleural effusion, 2 cases showed healed pulmonary primary complex; 2 showed thickened pleura and 1 showed old healed pleurisy with calcified lymph nodes.

Barium meal study of G.I. tract and barium enema study of Colon were done in only suitable cases and comprised 37 cases and 29 cases, respectively. In 31 cases there was positive evidence of obstruction or filling defects; with barium enema study 17 showed evidence of filling defect in the caecum and colon and 1 showed abnormality of mucosal pattern.

Investigations of relatives.—Relatives of 57 cases were investigated; 6 persons showed radiological evidence of tubercular disease of the chest.

Endometrial biopsies were examined for A.F.B. by smear and culture method and for histopathology in 16 married women. Histopathology of the biopsied lymph nodes was done in 57 cases—lymph nodes, Mesenteric-19, neck-25, axilla-5, inguinal-2, a piece of intestine-6.

Culture for A.F.B.—In all cultures were set up in 77 cases. The material employed was: mesenteric lymph nodes-19, lymph nodes neck-27, lymph node axilla-5, inguinal lymph nodes-2, intestinal wall mucosa-3, intestines plus mesenteric glands-6, ascitic fluid-5, pus from cold abscess-2, pus from emphysema necessitans-1, pleural fluid-1, CSF-3, menstrual blood-2, and joint fluid-1. Lowenstein Jensen with and without glycerol and chicken extract; Dorest egg media with and without glycerol were employed and 10 tubes were used for each case.

Out of these 19 gave positive cultures and one additional positive culture was obtained from gastric lavage. Forty-eight specimens were

also injected into guinea-pigs and acid fast bacilli were isolated from the viscera of 12 guinea-pigs. Out of these 12 positive cases, positive cultures from the material as well was obtained in 8 cases, whereas in 4 cases the culture was negative. The morbid material that gave positive growth was : Neck glands-11 cases, inguinal glands-1, axillary glands-1, mesenteric glands-4, mesenteric glands *plus* intestines-2, intestines alone-2, intestinal subserous tubercles-1, menstrual blood-1 and gastric lavage-1.

Typing of cultures.—Decision regarding the type of tubercle bacillus was made after observing the results of animal inoculation. However, cultural characters, medium of growth, type of colony, pigmentation, etc., were also duly noted. Both rabbits and guinea-pigs were injected with culture of each strain. Post mortem examination of each animal on death or on sacrificing after a lapse of 24 weeks or over was done. Viscera of these animals were examined carefully. Spleen, liver, lungs, kidneys, omentum bearing tubercles were taken out, put up for culture and histopathology.

Results.—Thirteen were human types, in 4 results were inconclusive as the animals did not develop the tubercular disease (possibly the effect of antibiotics given to the patients before the material was taken for culture). Three cases could not be proceeded with and 4 cases are pending at the time of writing, possible human types. These latter 4 cases produced extensive tuberculous disease both in rabbits and guinea-pigs. Animal inoculation was repeated in these 4 cases taking 3 rabbits for each strain.

109. Tuberculosis Chemotherapy Project, Madras, under Dr. P.V. Benjamin, Adviser in Tuberculosis, Directorate General of Health Services, New Delhi.

The work on the tuberculosis Chemotherapy Project set up at Madras by the Indian Council of Medical Research in collaboration with the W.H.O./B.M.R.C. was started in May, 1956. The study has been designed to yield information on the following points :

1. The relative merits of domiciliary and sanatorium chemotherapy.
2. The efficacy of several different regimes of domiciliary chemotherapy in comparison with each other, and, if indicated, with chemotherapy in sanatorium.
3. The extent to which the infectivity of groups of domiciliary patients can be reduced by chemotherapy.
4. The prevalence of tuberculosis in family contacts at the time of diagnosis of the index case and the subsequent incidence of tuberculosis amongst them.

5. The identification and virulence of the causative organisms and their comparison with strains of tubercle bacilli from England.
6. The relationship between the pathogenicity of isoniazid-resistant strains of tubercle bacilli and their ability to cause relapse in the patient and to infect contacts.
7. Some of the methodological factors involved in the mass application of chemotherapy (e.g. co-operation of patients and methods of sputum collection).

2. The requirements for the work are : Clinical, home-visiting, laboratory, x-ray, transport and administrative equipment and staff, and a supply of anti-tuberculosis drugs. The project is jointly staffed, equipped and supplied by the I.C.M.R., the Madras Government and the W.H.O./B.M.R.C.

3. The progress of the work on the Project is reviewed from time to time, by a Project Committee constituted for the purpose. During 1957, two meetings of the Project Committee were held, one in July and the second in November, 1957. Apart from the technical aspect of the project, the Committee considered certain administrative matters also e.g. staffing requirements, construction of buildings for the Centre, air-conditioning of the animal house provided for the scheme.

4. During the year 1957, a comparison of combined chemotherapy in domiciliary and sanatorium patients was undertaken and a further study of different forms of chemotherapy in domiciliary patients was initiated. In both studies the prevalence and attack rates of tuberculosis in the contacts are being investigated.

5. The laboratory has organized a culture, sensitivity test and catalase activity service, has introduced a number of tests to identify the causative organisms and has evolved a practical test for the detection of isoniazid in the urine. A breeding stock of guinea-pigs has been built up.

110. Inquiry to study the cultural characters and pathogenicity of chromogenic acid-fast bacilli and atypical strains of *Myco. tuberculosis* under Dr. Balbir Singh at the Irwin Hospital, New Delhi.

PLAN OF INVESTIGATION

1. To search for and study the characteristics of acid-fast bacilli causing disease indistinguishable from pulmonary tuberculosis.

2. To compare the above organisms with : (a) human strains of *Myco. tuberculosis* sensitive to PAS, streptomycin and isoniazid, (b) human strains of *Myco. tuberculosis* resistant to PAS, streptomycin

and/or isoniazid, (c) Acid-fast saprophytes present as cotaminants in cultures from sputum.

MATERIAL

1. 5 human strains for reference :— (a) $H_{37}R_v$, (b) 2 freshly isolated human sensitive strains obtained from France, (c) 2 freshly isolated human sensitive strains obtained from U.S.A.

2. 17 pre-treatment human strains, catalase-positive and sensitive to PAS, streptomycin and isoniazid.

3. (a) 3 Catalase-positive human strains resistant to isoniazid. (b) 2 catalase-negative human strains resistant to isoniazid.

4. (a) 1 human strain catalase-positive and resistant to PAS, streptomycin and isoniazid, (b) 3 pigmented strains received from N.D.T.B. Centre, and (c) 1 strain of the so-called yellow bacillus obtained from Dr. E. Nassau, Harefield Hospital, London.

RESULTS

Results were recorded in terms of Feldman index for gross pathology. Maximal values for extensive involvement of spleen, lung, liver and the site of inoculation and regional glands respectively were taken as 35, 30, 25 and 10. Cultures were set up from the tissues. Histological sections were studied and the results were recorded in terms of Feldman index. The maximal values for extensive involvement with progressive lesions in the organs and in the regional glands and the site of inoculation were the same as for gross pathology mentioned above. Total of maximal values was 100.

DISCUSSION

Of the 17 catalase-positive sensitive strains, only 4 showed nearly the same indices for gross pathology as were observed with the five reference strains, whereas the remaining 13 strains had comparatively lower indices than these and showed no increase in the average index for gross pathology when the guinea-pigs were allowed to live for 10 to 11 weeks instead of 4 to 6 weeks.

Catalase-positive and catalase-negative isoniazid-resistant strains showed low index for gross pathology and the results were similar to those already reported by other workers.

One non-pigmented and 2 pigmented catalase-positive strains resistant to all three drugs showed lesser pathogenicity than the reference strains. The disease did not spread beyond spleen even after 11 weeks of inoculation with 10^6 and 10^7 units of organisms, respectively of one of the pigmented strains (528). Though the other pigmented strain (2004) and the non-pigmented strain (434) also showed similar absence

of histological evidence of disease in liver and lungs, but both these produced micro-abscesses and tubercles in spleen of some of the guinea-pigs that were allowed to live for 14 to 15 weeks after inoculation.

CONCLUSION

A fair number of sensitive strains of *Myco. tuberculosis* isolated from patients suffering from pulmonary tuberculosis show lesser pathogenicity than is shown by freshly isolated American and French sensitive human strains. These findings are in accord with the work already reported by Dr. Frimodt Moller.

111. 'Studies in *Mycobacterium tuberculosis*' under Dr. Balbir Singh at the Irwin Hospital, New Delhi.

Plan of investigation.—To determine sensitivities of pre-treatment strains of *Myco. tuberculosis* isolated from patients suffering from pulmonary tuberculosis against PAS, streptomycin and isoniazid.

Material.—105 strains of acid-fast bacilli isolated from sputum of 105 patients who attended N.D.T.B. Centre for treatment of pulmonary tuberculosis for the first time.

Results.—27 patients (26 per cent) showed strains resistant to one, two or all three drugs. The number of patients showing strains resistant to isoniazid, streptomycin and PAS is 25 (24 per cent), 17 (16 per cent and 8 (8 per cent), respectively.

Comments.— It is very unlikely that all these 27 patients were infected initially with resistant strains and that resistance had not developed as a result of treatment. Sixteen patients were no longer traceable for further inquiry but their case-sheets showed that 4 had received chemotherapy and 8 had probably been treated with chemotherapy before they sought medical advice at the N.D.T.B. Centre. No information could be elicited about the remaining four. All these 16 cases though included in this inquiry cannot be considered for any statistical evaluation.

The clinical progress of 11 patients is, however, known. Three made no improvement, and one out of the remaining 8 had been treated with viomycin and PAS and not with streptomycin and isoniazid to which the strain isolated from his sputum was resistant. Seven patients who improved on treatment with streptomycin and or isoniazid showed strains of varying degrees of resistance to one or both drugs. Strains of only four patients were highly resistant to streptomycin, PAS and or isoniazid, whereas the strains of other three were on the border line of resistance. No conclusion, however, can be drawn from this small number of cases.

One significant feature of the results of sensitivity tests of the strains that showed resistance was that 6 strains were resistant to all three drugs. Two strains out of these were chromogenic.

112. Inquiry into the susceptibility of guinea-pigs from different parts of India to infection with *Mycobacterium tuberculosis* under Dr. R.K. Goyal at the S. M. S. Medical College, Jaipur.

Graded doses of tubercle bacilli were injected into batches of guinea-pigs which were sacrificed at regular intervals. The initial tentative plan suggested was to sacrifice all of them at the same time, say after 8 weeks. This plan was not considered suitable due to two reasons: (1) The animals injected with bigger doses of bacilli, say 1,000 or over died earlier in a number of cases, so the guinea-pigs obtained from different parts of India had to be sacrificed earlier to assess their relative resistance. The guinea-pigs injected with a small number of bacilli, say 5 to 10 did not show any appreciable lesions after 8 weeks, so they had to be sacrificed at a later period. (2) as the organs also had to be cultured for presence of tubercle bacilli, it was not convenient to put up cultures of a large number of organs at the same time.

The cultures of different organs from the point of view of determining the relative susceptibility of guinea-pigs from different parts of India did not give satisfactory results. Out of the cultures put up from the organs of 67 guinea-pigs, only five cultures were positive, these guinea-pigs had been injected with 100 to 100,000 bacilli. The smear examination of the inoculated media was positive in another 13 guinea-pigs, nine of which had been injected with 100 to 100,000 bacilli, and three only with ten bacilli. It is evident that when 10 to 100,000 bacilli are injected into the guinea-pigs the tubercle bacilli can be recovered only in a minority of cases.

For carrying out the animal experiment to determine their relative resistance, a fairly virulent strain of human tubercle bacilli was used throughout, 10 to 100,000 bacilli were used in ten-fold dilutions in different experiments, 2 to 4 guinea-pigs were injected with each dose. They were sacrificed at different periods depending on the dose injected, the earliest being 3 weeks and the latest 3 months.

To sum up, although a very large number of guinea-pigs were obtained from different sources, only 238 guinea-pigs could be used in the above-mentioned experiments. It could be concluded that the guinea-pigs varied appreciably in resistance from each individual source, but it was not clearly demonstrable that the resistance of the guinea-pigs from different sources did not vary markedly.

On making inquiries from different Institutes, the original source from which the guinea-pigs were obtained could not be ascertained from the Central Research Institute, Kasauli, Pasteur Institute, Coonoor and

the Armed Forces Medical College, Poona. The Indian Veterinary Research Institute, Muktesar, obtained its initial supply from Kasauli and Madras. There has been inter-breeding of guinea-pigs from different sources at the Haffkine Institute, Bombay, Central Drug Institute, Lucknow, and Pasteur Institute Coonoor, so it is not surprising that the guinea-pigs have varied appreciably in their resistance.

113. Studies on the nature of drug resistance in *Mycobacterium tuberculosis* H₃₇R_v under Dr. M. Sirsi at the Indian Institute of Science, Bangalore.

While the spectacular advances in the chemotherapy of tuberculosis has considerably reduced the mortality, it has not been able to eradicate the disease completely from the system. An understanding of the nature of the drug resistance and the host parasite relationship is essential for further progress both in conferring resistance to the host and the development of new chemotherapeutics.

M. tuberculosis has been shown to grow best in a medium where the source of carbon is supplied in the form of glycerol. Less amount of growth takes place if glucose is supplied as the source of carbon. This is rather characteristic of *M. tuberculosis*, among the various micro-organisms and might mean that *M. tuberculosis* is deficient in some of the enzymes, which, in other organisms, carry out the conversion of glucose to glycerol through the intermediates glucose 6-phosphate, fructose 6-phosphate and fructose 1, 6 diphosphate. To clarify this, among other things, it was decided to study the carbohydrate metabolism of *M. tuberculosis* both with glycerol and with glucose as the carbon source. Glycerol was tried first.

The basis of chemotherapy lies in the use of anti-metabolites for the substrates of those enzymes which are responsible for carrying out the various metabolic reactions in the cell of the disease-carrying organism. Of these, various metabolic pathways, carbohydrate metabolism is the most important as it gives rise to intermediates in the synthesis of proteins, fats, nucleic acids, porphyrines, etc.

Development of drug resistant mutants from the parent strain Myco. tuberculosis H₃₇R_v.—The process of inducing the resistance is, with certain drugs, very slow and time-consuming. Nevertheless, the resistant mutants developed in the laboratory were preferred to those isolated from patients treated with the drugs, since a comparative evaluation with the parent strain could be carried out only with the former method.

Mutants resistant to INH (100 μ), Dihydrostreptomycin (1000 μ) and PAS (1000 μ) have been developed on a solid medium (Petri medium) and some of their biochemical activities and pathogenicity studied.

Standardization of the paper chromatographic technique for the isolation of sugars.—Recently, paper-chromatographic technique has come

into vogue for biological work for the separation as well as quantitative estimation of sugars. U-Butanol : pyridine : water 10 : 3 : 3 (v/v) solvent system at room temperature, double development with circular paper-chromatographic technique and identification by the benzidine trichloroacetic acid spray reagent were used for the separation and identification of most of the sugars. Preliminary work indicated that glucose, galactose, mannose and arabinose are formed during the metabolism of *Mycobacterium tuberculosis* H₃₇R_v. While galactose and glucose were easily separated by the above solvent system mannose and arabinose, however in view of their very close R_f values could not be separated. To separate these sugars a number of solvent mixtures in different proportions, viz. n-butanol : acetic acid : water ; n-butanol : ethanol : water ; n-butanol : acetone : water ; ethylacetate : acetic acid : water ; ethylacetate : pyridine : water ; phenol : water (to mention only a few), were tried using the circular paper-chromatographic technique at three different temperatures, viz., 60°F., room temperature, and 37°C. No solvent under any of these conditions was found to give clear separation. Hence, two dimensional paper-chromatographic technique was resorted to with beneficial results.

Qualitative studies on the carbohydrate metabolism of M. tuberculosis H₃₇R_v.—With glycerol as the source of carbon, *M. tuberculosis* was grown in modified Youmans' medium and harvested at definite intervals. Culture filtrates, cell homogenates and their hydrolysates were prepared and the qualitative analysis of the sugars of these fractions gave the following results.

Culture filtrate.—This fraction did not give any indication as to the changes that take place in the composition of sugars during the metabolism of the tubercle bacilli with glycerol as the source of carbon. However, the culture filtrate hydrolysate showed that mannose, arabinose, glucose and galactose are present after 11 days in quantities detectable by this method.

Acetone and TCA extracts.—These extracts indicated that acetone and 5 per cent TCA not only killed the cells but also extracted some of the sugars and polysaccharides which were found to be formed in the cells after 2 weeks.

Cell homogenate.—This fraction showed that after two weeks some polysaccharides were formed in the cells in quantities detectable by this method. Hydrolysis gave mannose, arabinose, glucose and galactose.

Cell hydrolysate.—Like cell homogenate and its hydrolysate this also revealed the presence of mannose, arabinose, glucose and galactose.

114. Pulmonary function studies in healthy adults and in those with pulmonary tuberculosis under Dr. H. B. Dingley at the Tuberculosis Hospital, Mehrauli Road, Delhi.

Pulmonary-function studies, mainly ventilatory, have been carried out in a group of 50 healthy adults, males and females, known to be free

clinically and radiologically from pulmonary and cardiocirculatory diseases. Measurements of vital capacity, inspiratory capacity, inspiratory reserve volume, expiratory reserve volume and maximum breathing capacity were done.

The figures were compared with the predicted values based on regression formulæ taking into consideration, age, weight, height and body surface area. Figures were obtained both in the supine and standing position. The determinations were done by recording on a spirometer of the closed circuit type, derived from the Benedict-Roth apparatus in general use for basal metabolism determinations with some modifications

Measurements were recorded at body temperature ($37^{\circ}\text{C}.$) prevailing barometric pressure and saturated with water vapour (B. T. P. S.). Each spirometer has its own calibration factor to convert millimeters to cubic centimeters. The temperature of collected gas has a correction factor, both these are put together for getting the final figures.

CONCLUSIONS

The work is still in progress and the determination of the functional residual air and the total capacity of the lung is being carried on, but the results so far obtained are:—

- (1) Vital capacity decrease with increasing age and increases with increasing height, but it has no definite relationship with weight.
- (2) Vital capacity is lower in the supine position as compared with standing position. This is largely due to difference in pulmonary blood volume.
- (3) The inspiratory capacity range is 70 to 80 per cent of the vital capacity and expiratory reserve volume is 20 to 25 per cent.
- (4) The maximum breathing capacity in standing position is 3 to 5 per cent greater than the supine position.
- (5) A high positive correlation exists in normal males as well as in normal females between maximum breathing capacity and vital capacity.

115. Inquiry on Bronchoscopic studies in cases of pulmonary tuberculosis under Dr. R. N. Tandon at the Kasturba T. B. Clinic, Lucknow.

Bronchoscopic studies were done in 19 cases of pulmonary tuberculosis proved on clinical, radiological and bacteriological evidence. Fourteen cases were males and 5 females. This ratio represents the distribution of beds in our Hospital.

I. OBSERVATIONS

(1) *Clinical evidence of endobronchial disease.*—A clinical evaluation of endobronchial disease was made in each case before bronchoscopy.

Clinically, endobronchial disease was present in 9 cases and the findings were :—

- (i) Localized wheeze.
- (ii) Tension cavity.
- (iii) Atelectasis.
- (iv) Ballooning and frequent alteration in the size of the cavity.

(2) *Bronchoscopic findings.*—Bronchoscopic evidence of endobronchial disease was present in 12 of the total 19 cases. Bronchoscopic findings were classified according to the criteria recommended by the Research Committee of the British Tuberculosis Association into three groups :—

- (i) Minor mucosal changes (including redness and easy bleeding points)--1 case.
- (ii) Gross mucosal changes (including oedema, ulceration and granulations)—10 cases.
- (iii) Stenosis—1 case.

This classification was found suitable for our purpose upto now.

(3) *Bacteriological examination.*—Specimens of secretions by bronchoscopic aspirations were examined bacteriologically (smear examination) for acid fast bacilli in all the 19 cases. A. F. B. could be demonstrated by this method in 14 cases.

(4) *Complications.*—Except for the increase in intensity of cough for a few hours after bronchoscopy in 2 cases, no other complications were noticed.

II. RESULTS

(1) The number of cases done are too small to assess the incidence of endobronchial disease in pulmonary tuberculosis. It will be possible to do the same in the final report when more cases will be studied.

(2) A smear examination of the sputum in 19 cases showed A. F. B. in 11 cases only ; 8 cases were negative. Smear examination of the specimens of bronchoscopic aspirations was positive in 14 cases. In 2 of the 5 cases which were negative, endobronchial disease of minor degree (1 case) and gross (1 case) was present. A culture examination of the specimens have been done, the results are not yet available.

(3) Out of the 9 cases in which the clinical evidence of endobronchial disease was present, bronchoscopy revealed endobronchial disease

in 7 cases only. In the other two cases with acalectasis (1 case) and tension cavity (1 case) as radiological evidence, only mucous plug was present on bronchoscopic examination. In 5 cases endobronchial disease was present but there was no clinical evidence of the disease.

(4) No complications, such as fever, spread of disease, tubercular laryngitis, atelectasis, bronchitis or bronchospasm, were noticed in the present series.

116. Diagnosis of active primary pulmonary tuberculosis in children below the age of 7 years under Dr. Saktipada Bhattacharjee at the Nilratan Sircar Medical College and Hospital, Calcutta.

I. One hundred cases of primary pulmonary tuberculosis in children below 7 years of age have been studied. They are undergoing treatment with specific anti-tuberculous drugs and kept under observation. Physical, laboratory and radiological examinations were carried out in these cases to establish diagnosis. The economic state and the environments have been studied and a thorough search for contact made. The culture of material from gastric lavage has been started recently. Radiologically these cases were grouped as follows :—

- | | |
|---|--------------|
| A. Active primary pulmonary tuberculosis—presence of glandular and/or lung component | ... 12 cases |
| B. Early progressive primary pulmonary tuberculosis—evidence of progressive glandular and/or that of the parenchymal lesion | ... 70 cases |
| C. Progression of stabilized primary complex at a later date | ... 8 cases |
| D. Evidence of hæmic and lymphatic spread | ... 7 cases |
| E. Healed primary pulmonary tuberculosis, disappearance and/or calcification of components parenchymal and glandular | ... 3 cases |

Tuberculin test has been carried out in all 100 diagnosed cases of active primary pulmonary tuberculosis with old tuberculin solutions of varying dilutions starting from 1 in 10,00,000 to the dilution of positive reaction using Mantoux method.

Injections of different strengths (freshly prepared with normal saline) of old tuberculin are being given every 4th day in alternate forearm to the negative reactors.

Other laboratory examinations, e.g. estimation of E.S.R., total and differential counts of white blood cells culture of gastric lavage material and smear examination of sputum have been carried out.

II. Specific anti-tuberculous drugs were administered in all cases that could be kept in touch with the working unit. They have been grouped as :—

- (i) Cases treated with isoniazide and PAS.
- (ii) Cases treated with streptomycin and PAS.
- (iii) Cases treated with streptomycin and isoniazide.

Follow up.—Fifty cases, in total, had been periodically checked up at intervals of threemonths clinically and by ESR, skiagram and graded tuberculin reaction by Mantoux method.

A separate group of 40 cases suspected to be suffering from primary pulmonary tuberculosis from history of illness, history of contact and by clinical examination, etc., were kept under observation though they revealed no focus of infection radiologically.

Tuberculin test was positive in 20 cases. Re-X-ray after three months revealed lesions in lung in three cases (tuberculin test positive) and they are having treatment accordingly and will also be followed up by periodical check up.

117. Inquiry on effect of antitubercular drugs on the synthesis of phthioic acid in mycobacterium tuberculosis under Dr. S. Chandrasekhar and Lt. Col. A.J.H. de Monte at the Vallabhbhai Patel Chest Institute, Delhi.

The strain $H_{37}R_v$ was made resistant to streptomycin, one microgram/c.c., 10 ug./c.c. and 100 mg./c.c. on Lowenstein Jensen medium. These resistant strains together with $H_{37}R_v$ sensitive strain to serve as a control were employed for the estimation of their phthioic content to determine what changes in the phthioic content of $H_{37}R_v$ strain occur as a result of acquired streptomycin resistance at varying levels of resistance and the result compared with the phthioic-acid content of the parent sensitive $H_{37}R_v$ strain.

Various media were tried out to determine the best that would give a maximum growth and finally Youman's medium containing 40 g./litre glycerol and ferric ammonium citrate 0.05/ litre was selected. 500 c.c. of this medium in 3-litre flasks were inoculated separately with the resistant and control strains. The inoculating dose was 2 mg./c.c. Incubation was at 37°C. for 42 days.

Isolation of phthioic acid.—Phthioic acid was isolated from wet tubercle bacilli by the method communicated personally to us by J. Cason and which is as follows:

1. The culture was sterilised by autoclaving at 100°C. for 3 hours.
2. The cells were extracted with ether and alcohol several times.
3. The lipids were saponified by refluxing with alcoholic KOH and the fatty acids separated from carbohydrates and other insoluble material.

4. The mixture of fatty acids was esterified with methanol sulphuric acid (5 per cent). This esterified the alpha-beta unsaturated acids and acids with a large substituent in the position.

5. The fatty acids were extracted with alkali and recovered by precipitation with H_2SO_4 (5 per cent and extraction with ether).

The results show that total lipids, fatty acids, alpha-beta unsaturated acids increased in the resistant strains as compared with the controls. The values of C_{27} phthienic acid decrease with resistance.

118. Inquiry on the influence of nutritional factors on tuberculin reaction in experimental tuberculosis under Dr. M. Sirsi at the Indian Institute of Science, Bangalore.

Using old tuberculin the quantitative evaluation of tuberculin reaction on guinea-pigs infected with virulent strain ($H_{37}R_v$) on *Myco. tuberculosis* has been standardized.

Utilizing the technique, it has been shown that certain lipids interfere with the manifestations of tuberculin hypersensitivity. Amongst the lipids tested, it is noticed that neem oil, when injected subcutaneously, considerably reduces the tuberculin hypersensitivity; the pea-nut oil lessens the reaction to a slight extent, while coco-nut and gingelly oils exhibit no influence.

119. Inquiry into morbid anatomy and bacteriology of resected lungs in pulmonary tuberculosis under Dr. D. Barua at the Institute of Post-Graduate Medical Education and Research, Calcutta.

Procedure of study.—Lobes or segments of lungs removed from cases of pulmonary tuberculosis who have had a fairly long course of antibiotics and chemotherapeutic agents are collected aseptically in the operation table and are sent to Department of Pathology & Bacteriology. The specimen is studied with all aseptic precautions. After noting the size of the specimen and condition of the pleura, the bronchi are traced, as far as possible, and the lesions are exposed. They are then classified into different types. The condition of the parenchyma and bronchi are also noted. Material is collected from different lesions for bacteriological investigations.

When no acid fast bacilli are demonstrated by ordinary microscopical examination or by fluorescent microscopy, half of the specimen is subjected to homogenization in a tissue homogenizer and the homogenate is subjected to bacteriological investigations.

Bacteriological study : investigation for acid fast bacillus.—Material collected from different types of lesions is examined immediately by smear preparations. Fluorescent microscopy is used when ordinary smear examination fails to show A.F.B.

The material is then cultured in about four to eight tubes of Lowenstein Jensen media and incubated at 37°C. They are observed for about 3 to 4 months before finally declaring them as negative in view of several recent reports about the late appearance of growth of tubercle bacilli from treated cases. In this series growth has appeared in most of the positive cases within two weeks.

The materials are also inoculated directly or after concentration into guinea-pigs weighing about 300 g. Guinea-pig inoculation tests are also done with the culture material when the test by injection of the material directly from the lung fails to give positive result.

Drug sensitivity of the isolated strains against streptomycin, INH and PAS is tested. The material is also investigated by direct microscopy, culture in blood agar and Sabouraud's maltose agar media for secondary organism including fungi.

Result of bacteriological study.—Specimen of lungs of 27 cases have so far been studied.

Microscopical examination.—In 6 out of these 27 cases, direct microscopic examination and fluorescent microscopy have failed to demonstrate A.F.B. in their lesions. No acid-fast bacillus could be demonstrated by smear, culture and animal inoculation in the lesions of the case No. 21. *M. tuberculosis* could be isolated from the lesion of case No. 13 by cultural examination and by guinea-pig inoculation though no A.F.B. could be demonstrated by microscopic examination of smear. In the remaining four (cases Nos. 6, 7, 14 and 15) smear negative cases, no A.F.B. could be isolated by cultural examination. Unfortunately, animal inoculation was not done directly with the material.

Cultural examination.—So far *M. tuberculosis* has been isolated from 8 out of 27 cases. Fourteen smear positive cases have failed to grow in the culture media and only one out of the six smear negative cases have grown in the culture media.

Animal inoculation test.—This test was not done in all the cases for unavoidable reasons in the beginning of this investigation. So far, it has been done in 19 cases, out of which 7 cases have definitely proved to be virulent. These seven cases include one smear-negative and two culture-negative cases. Three cases (cases Nos. 1, 12 and 25) grew in the culture media but failed to produce any lesion in the guinea-pig. Of the 12 guinea pig inoculation-negative cases 8 were smear-positive.

It appears from the preliminary study that out of 17 smear positive cases 8 failed to grow in the culture media and failed to produce any lesion in the guinea-pig. So, the acid fast bacilli in these 8 cases may be considered as non-viable organisms because all attempts to grow them in the culture media and in the susceptible animals have failed. These 27 cases also include 3 strains which grew in the culture media but failed to produce any lesion in the guinea pig (viable but avirulent).

No secondary organism or fungus have so far been isolated from the closed tuberculous lesions. Resident flora of the upper air passage are encountered occasionally in the open lesions.

Histological study.—Tissues are collected from the different lesions and their neighbourhood. At least 4 to 10 blocks are prepared from each specimen depending upon the types of lesions. The sections are stained by routine H & E, Mallory, and Ziehl Neelsen stain.

Histological findings.—There is a good deal of variation from case to case. Modified lesions of different types in different combinations are found. Attempts are being made to correlate the different types of lesions with the bacteriological findings and with duration of illness, amount of antibiotics given, etc.

It is not yet possible to try to find any correlation. Large number of cases, however, show signs of active lesions in spite of prolonged treatment with antibiotics. Some of the findings are different from those that have been reported by workers from other countries. This may be due to the fact that the cases of this series were treated as domiciliary cases and the antituberculous regime has not been as rigid as in other countries. This investigation with resected lung specimen is being continued.

Attempts will now be made to produce experimental tuberculosis in animals and to see the effect of different antibiotics on their lesion and to produce tuberculosis in animals with the organisms which have proved to be avirulent by lowering the resistance of the animals.

120. Contact examination and treatment with isoniazid of Mantoux positive children under Dr. M.D. Deshmukh at the T.B. Clinic, J.J. Group of Hospitals, Bombay.

1. Total number of 414 individuals from 71 'contact' and 'non-contact' families have been x-rayed and Mantoux tested from January 1957 to September 1957.

2. Mantoux was positive in 14 out of 16 children (87 per cent) from 'heavy contact' families, in 124 out of 142 children (88 per cent) from 'not-heavy contact' families and in 53 out of 71 children (75 per cent) from 'Non-contact' families.

3. Hilar glands enlargement was seen in 14 out of 17 children (82 per cent) from 'heavy contact' families. 106 out of 147 (72 per cent) children from 'not heavy contact' families and 56 out of 79 (70 per cent) children from 'non-contact' families. There were altogether 4 parenchymal lesions 1 from 'heavy', 2 from 'not-heavy' and 1 from 'non-contact' families. The lesions from 'not-heavy' and 'non-contact' families were of a primary type, while one seen in child of 'heavy-contact' family was of a chronic adult type of lesion.

4. Forty-one children getting I.N.H. therapy have been re-tested and show the following changes — 2 showed reversal of Mantoux reaction, 6 showed diminution of degree of reaction and in 33, there was no change.

5. Observations have been extended to the babies, born of tuberculous mothers, who will be divided into three groups—one on I.N.H., one on B.C.G., and one on I.N.H. after Mantoux conversion by B.C.G.

121. Clinical and pathological study of tubercular lymphnode enlargement under Dr. S.P. Srivastava at the Medical College, Agra.

Material.—Clinically diagnosed tuberculous adenopathy attending the Out patient and Inpatient departments of the S.N. Hospital, Agra, have formed the subject of the study.

After a thorough clinical examination of these cases and after a few preliminary investigations, one of the enlarged lymphnodes or the pus from the cold abscess is collected for the purpose of further histologic examination, guinea-pig inoculation and for culture on special media, with a view to confirm the diagnosis and also to see whether the T.B. bacilli causing this lymph adenitis belongs to the human or bovine variety.

Observations and results.—In 165 cases (110 in 1956 and 55 in 1957) examined and followed by us, a few important and interesting findings were observed. Tuberculous lymphadenopathy is essentially a disease of first two decades of life (72 per cent). Up to 20 years of age, 11 to 15 years and 16 to 20 years age groups are affected maximally. The disease becomes rare with the advancing age and no case was recorded above 40 years of age except two (1957) who were 65 years old. None of the workers have recorded tubercular lymphadenopathy over the age of 60 years except Thompson (0.3 per cent).

Sex distribution.—Disease was found to be more common in females, e.g. 69 out of 110 cases in 1956 and 37 out of 55 in 1957.

Caste and distribution.—The disease is more common in Muslims and more so in females. As far as diet is concerned, no important conclusion could be drawn as the patients of this inquiry had occasional changes to non-vegetarian diets though basically vegetarian.

Economic status.—The inquiry revealed that nearly 70 per cent cases had an income of Rs. 30 per month per capita. Only 5 per cent of the cases had a per capita income of over Rs. 50 per month.

Previous history.—33 cases of the series gave definite history of contact with pulmonary tubercular patients and 20 cases gave history of using unboiled milk for more than 6 months.

Groups of lymphnode enlargement.—Mostly cervical nodes were enlarged. The other groups involved were axillary—30 in 1956 and 11 in 1957, inguinal—18 in 1956 and 1 in 1957, and mesentric 7 in 1956 and 2 in 1957. Only five (3 in 1956 and 2 in 1957) cases had generalized lymphnode enlargement involving more than three sites in the body.

Classification.—A practical, easy and quick classification of the tubercular lymphnode enlargement was evolved by us.

	1956	1957
Stage I. Non-matted discrete glands	43	17
Stage II. Matted glands	16	28
Stage III. Matted glands with cold abscess	7	2
Stage IV. Glands with sinuses and ulcers	17	8

The series shows that the maximum number belongs to stage 1 though disease was present for many months.

Histological Studies.—These were made in 42 cases in 1956 and 27 in 1957. Out of these cases lesions were found to be in 31 in 1956 and 22 in 1957, while the hyperplastic series were found in 2 (1956) and 5 (1957) A.F.B. stain revealed the presence of tubercular bacilli in three out of these cases (1957).

Culture examination.—Eleven cases were examined by inoculating gland suspensions or pus from cold abscess during the year 1956.

The media tubes have been inoculated and it requires minimum period of six weeks to culture the organisms before some helpful results are expected.

Bacteriological studies.—In the year 1956 we have inoculated 11 guinea-pigs. This year we have inoculated three guinea-pigs and are still continuing the work.

VENEREAL DISEASES

122. Inquiry on treponemal agglutination test (TPA test) for syphilis under Dr. C.W. Chacko at the Institute of Venereology, Madras.

In the progress reports submitted up to September 1956, it had been stated that the TPA test for syphilis, using Nichols strain of virulent *T. pallidum*, as a killed agglutinating antigen suspension, appeared not to have any advantage over the three standard routine serological tests, namely the Wassermann, the Kahn and the VDRL tests for syphilis from the point of view of sensitivity and specificity in syphilis when they were all tested in parallel on a significant number of specimens of

Sera of syphilitic and non-syphilitic origin. The potential specificity of the test had, however, seemed to manifest when the test was modified to a test after-absorption-technique in which the non-specific Wassermann antibody was first absorbed and the TPA test was then performed. When this was done in known syphilitic sera, on a significant number of specimens, the TPA test continued to be positive. This had pointed to the possible presence side by side, in syphilitic sera, of the agglutinating non-specific Wassermann antibody and a agglutinating specific treponemal antibody. To prove this conclusively, in the group of non-syphilitic sera reacting falsely to the routine standard serological test for syphilis, the Wassermann antibody alone should be present and the TPA test performed after the absorption of the Wassermann antibody must be negative. Significant number of unequivocal and suitable specimens of sera of this false reacting category have not been available. A good many of them investigated have been comparatively of low titre (less than 4 dils). The technique of absorption of the Wassermann antibody was such that in these low titred group, the 'dilution factor' involved in mixing of the tissue extract antigen with the sera alone could explain the negative reaction to the TPA test-after-absorption. Under these conditions, it was felt that the TPA test-after-absorption technique, may not be a practical procedure worth spending any more efforts for the present in this inquiry. The whole series of results, obtained with the TPA test, are being analysed and conclusions drawn are to be finally reported in due course. During the period of this review, in place of the TPA test, alternative procedures involving the use of antigen of virulent treponemal origin which have been recently reported favourably by workers from abroad in their preliminary trials, have been investigated. These tests have been *T. pallidum* complement fixation (TPCF) test (Magnuson 1956 ; Price 1957), *T. pallidum* immune adherence (TPIA) test, (Nelson 1952 ; Daguet 1956), and *T. pallidum* hæmagglutination (TPHA) (Daguet 1956) test for syphilis.

Preliminary attempts to reproduce and establish the TPCF tests, have not been successful as the virulent *T. pallidum* antigen in the form of whole organism and in the cryolysed form, would not fix complement in the presence of specific treponemal antibody.

The TPIA-test technique has been reproduced and established successfully and is now under evaluation of its reported specific value in syphilis.

The TPHA-test procedure has shown itself on reproduction, to be a comparatively more difficult technique than the TPIA test but as a interesting procedure in the field of syphilis serology, it is under our further examination.

TPIA-TEST FOR SYPHILIS :

Principle of the test.—When syphilis serum containing specific treponemal antibody is mixed with a suspension of killed virulent *T. pallida*, human red blood cells and fresh complement and incubated, the *T. pallida* attach themselves to the r.b.c. specifically and disappears from the supernatant of the lightly centrifuged mixture of the reagents.

TPIA-antigen.—It consists of a suspension in saline of killed Nichol's strain of virulent *T. pallida* grown and extracted from live rabbit testes. Actually, the TPIA antigen has been the same as the TPA antigen which is diluted to contain 15 to 20 *T. pallida* instead of 50 to 60 per dark field in the latter.

Complement.—Source has been pooled guinea-pig sera from freshly drawn blood from the heart.

Red blood cells.—Human '0' group cells, received citrated from the blood bank and washed repeatedly and used in 50 per cent suspension in normal saline.

The test technique.—0.5 c.c. of the antigen suspension is mixed with 0.1 c.c. of the inactivated test serum, and 0.25 c.c. of Hanks solution (buffered saline) in test tubes and incubated in a water bath at 37°C. for 30 minutes. Then 0.05 c.c. of complement and 0.1 c.c. of r.b.c. in 50 per cent suspension is added and again incubated under the same condition for another 30 minutes. Controls consisting of known positive syphilis and negative sera and controls for each of the other reagents were included in the test under the same conditions of the experiment. The tubes were then centrifuged at 500 r.p.m. for five minutes and 0.05 c.c. of the supernatant was transferred to slides with the Kahn pipette. Dark-field examination of *T. pallida* were then done under high dry objective of the microscope and the number in 20 consecutive fields were counted. The percentage of organisms specifically adhered and disappeared with red cells were calculated as follows :—

Specific disappearance per cent

$$= \frac{\text{T.p. antigen} - \text{T.p. test proper}}{\text{T.p. antigen blank}} \times 100.$$

The percentage disappearance above 50 was considered qualitatively positive provided the controls were valid.

123. Study of incidence and relationship of prenatal and neonatal syphilis abortions of still-births neonatal deaths and congenital syphilis and results of treatment with penicillin at various stages under Dr. B.B. Gokhale at the B.J. Medical College, Poona.

All the antenatal cases are divided into 2 categories : (i) Sero-positive, (ii) sero-negative. Every sero-positive case (quantitative test) is clinically examined and an attempt has been made to elucidate as complete and reliable history as possible. Information about previous anti-syphilitic treatment is collected. Only those sero-positive mothers who did not receive any anti-syphilitic treatment with penicillin in previous deliveries are studied and have been treated with penicillin alone. Simultaneously, another group of sero-negative cases with similar age, parity and term of pregnancy has been separately studied. This is termed as 'Identical cases' or Matching group. Both the groups

are followed to the maternity wards and the outcome studied. It has been necessary to follow this method in the assessment of value of penicillin treatment in prenatal syphilis as expectant mothers with positive S.T.S. cannot be kept untreated and thus control group of untreated cases cannot be had.

Babies of sero-positive treated mothers are examined clinically and serologically (quantitative test) at delivery. Blood of sero-positive mothers and babies re-examined, about 8 weeks after delivery; and both of them are clinically re-checked. Such a procedure helps in assessing the value of penicillin treatment in mother and its effects on the offsprings also. By this procedure it is also possible to differentiate between pre-natal syphilis and passive reaginæmia.

Working on these lines, 1,926 cases were examined antenatally at the Sassoon Hospitals. Poona; and 220 were found to be positive. Out of these 220; only 104 could be studied; as the remaining 116 could not be followed to their delivery as either they were non-co-operative (refused to come for the treatment and antenatal check-up) or left this station.

Out of 104 cases studied, 61 were positive and 43 were weakly positive (by V.D.R.L. test). Twenty-nine women out of this group have delivered recently and are under observation.

Results of the follow-up of the remaining 75 cases.

(P = Positive and WP = Weak Positive)

Followed number	Completed	Out of station (Left after delivery)	Cannot be traced	Non-co-operative, refused to give second sample
P 43	26	7	4	6
WP 32	13	6	4	9

A study of these 26 completed positive cases shows that all the children excepting one are clinically and serologically healthy and normal. This case had a rash on the body, but that was not syphilitic. The baby was serologically negative and otherwise healthy. Three children from the identical or matching group have expired; other children are normal.

Out of 13 cases of the weakly positive group, 4 have been treated because of their histories and/or because of the positive serological findings of their partners. All second blood samples of the babies are all negative, and they are all living and healthy. All the children from the identical or matching group are also normal.

It would not be right to draw any conclusions with this scanty data on hand.

124. Inquiry on the isolation and establishment of virus of lympho-granuloma venerum (LGV) in embryonated chicken egg and the preparation of Frei antigen from it under Dr. C.W. Chacko at the Institute of Venereology, Madras.

There is a great demand for Frei antigen in this country. Frei antigen of human bubo pus origin is the only type available from our own resources, and the suitable specimen of pus is available for preparation in too limited a quantity. The object of this inquiry has been to isolate a local strain of LGV virus and/or a standard strain of the virus, obtained from abroad in a lympholised state, and establish it in the yolk sac of embryonated chick eggs. Once it is done, the Frei antigen of yolk sac origin (Lygranum-Squibb) Type is to be prepared for a pilot study of its worth and then prepare it on a larger scale to meet all the demands for Frei antigen in the V.D. clinics of the country.

Materials and methods.—These have been essentially the same as detailed in the progress report on the inquiry submitted in September 1956 (*vide* ICMR Technical Report of the SAB 1956 p. 149).

During the period under this report additional 21 samples of human bubo pus from clinical LGV cases have been dealt with. Potent samples of Frei skin antigen were prepared from 13 of these by Frei's standard method of preparation. Out of these, 8 specimens of pus were experimented upon for the possible isolation of a local strain of LGV virus by inoculating intracerebrally into the yolk sac of embryonated chick eggs and in 30 day-old-white Swiss mice, at the same time, by the standard techniques described by other workers in the field and experimental modifications. It has not been possible yet to isolate a local strain.

In order to establish at least a standard strain of the LGV virus. 2 samples each of the standard J.H. strain from Dr. Barwell of London and KAM strain from Dr. Levine of Paris have been obtained and attempts to revive them in the yolk sac of the chick embryo according to directions of the above doctors have also been unsuccessful.

A strain of the related Psittacosis virus received from Dr. Srivastav at Nagpur and successfully established in the chick embryo yolk sac and mouse brain during 1956, is being maintained successfully. A sample of Frei type of skin testing antigen has been prepared from the Psittacosis strain, according to the technique described by Grace *et al* (1940). Preliminary clinical trials with Psittacosis virus as cross reacting antigen in skin tests in the diagnosis of LGV cases, are now going on.

Although, strictly not within the object of the LGV virus inquiry, experimental trial and practice have been made on the technique of inoculating embryonated chick embryo by the chorio-allantoic route. We have successfully isolated a strain of the virus of herpes simplex from progenitalis herpes case, primarily in the rabbit cornea. It is being maintained by sub passage in the chick embryo and mouse brain.

DENTAL HEALTH

- 125. Study of development and growth of dentition of Indian children under Dr. R.S. Nanda at the Dental College and Hospital, Lucknow.**

The project envisages a study of the growth and development of dental and oral tissues. The data pertinent to Indian populations is extremely meagre in this respect. For better understanding of the normal and abnormal in the fast changing individuals of 5 to 15 years of age, this study was started. Since the investigation is cross-sectional in nature, it was proposed to examine 5,000 individuals of both sexes, so that there are about 100 children in each of the years between 5 and 15.

An elaborate form for recording the data was prepared and the data are being compiled. Care is being taken to take children of average socio-economic level so that the sample is most representative. Each examination which includes a detailed health and dietary history, thorough clinical examination of oral tissues, full-mouth x-rays, and impression taking for the preparation of dental casts required on an average of 45 minutes for each child. 500 children have already been examined in this manner. The project is expected to be completed in 3 years. The first two years will be for the collection of data, while the last one will be utilized for its analysis.

- 126. Studies on human saliva under Dr. K.L. Shourie at the Sir C.E.M. Dental College, Bombay.**

Saliva plays a very important role in the health as well as disease of oral cavity and among Indians periodontal diseases are more prevalent as compared to dental decay. In this connection 2,000 children have been examined. As the Research Assistant (Chemist) sanctioned for the scheme could not be appointed so far the Laboratory part of the work has not been taken up.

- 127. Studies in the relationship of systemic and periofontal diseases (Diabetes mellitus and periodontal diseases) under Dr. K. L. Shourie at the Sir C.E.M. Dental College, Bombay.**

This study was taken up with a view to study the exact correlation of diabetes with periodontal structures. Patients attending the diabetic clinic, J.J. Hospital, were the subject of this study. About 150 patients have so far been investigated. Every patient's family history and past history, general as well as dental, history of diabetes, its onset, progress signs and symptoms were noted. Urine sugar, and other laboratory tests were also carried out. A thorough oral examination together with radiological examination was carried out. In this, colour, consistency, surface texture of the gingiva and oral mucosa was noted down. Pocket depths of the gingival sulci were also recorded. Alveolar bone condition could be observed from the full mouth X-rays. The data collected so far has to be compared with similar data from normal persons.

128. Inquiry into the effect of sodium-hexa-meta phosphate in the treatment of periodontal diseases under Dr. K.L. Shourie at the Sir C.E.M. Dental College, Bombay.

The object of the inquiry was to investigate whether the use of sodium-hexa-meta-phosphate would help in prophylactic or curative treatment of periodontal disease or not.

About 2,000 school children were examined for evidence of periodontal disease and the oral health recorded according to Russel's index. Upper and lower anterior teeth and upper and lower teeth of one posterior side of every child was scaled and polished and each one was given sodium-hexa-meta-phosphate for rising twice a day for about one month. After this period the oral health was again recorded together with the estimation of oral hygiene. During this examination it was noted that the Russel's periodontal score as well as the oral hygiene score was less on the side which was scaled. Whether this is due to the scaling and polishing or due to the rinsing carried out daily with sodium-hexa-meta-phosphate is not yet estimated.

129. Inquiry on study of blood changes associated with periodontal disease under Dr. T.N. Chawla at the K.G. Medical College, Lucknow.

Procedure of work.—Patients attending the Dental Out-patient's Department and the dental and medical students formed the subjects of this study. In all, 75 normal individuals have so far been investigated. A good periodontal and general systemic history is being taken. Cases where there were no detectable clinical signs of periodontal disturbances and free from any systemic disorders have been taken to constitute as normal individuals for purposes of this investigation.

Techniques employed.

(a) *Hæmatologic techniques.*—All estimations were carried out within four hours of the withdrawal of blood from the antecubital vein into Wintrobe's double oxalate mixture. Red cells were enumerated in Thoma's pipette in a dilution of 1 in 200, using isotonic sodium chloride solution as the diluent. Two pipette, two chamber method was employed as advocated by Ham and the standard error of the red cell count was determined in each case. Total leucocytes were counted in a dilution of 1 in 20 using 3 per cent acetic acid as the diluent. Hæmoglobin determinations were performed by acid-hæmatin technique of Sahli. For the study of red cell morphology and differential leucocyte counts, peripheral smears were drawn on glass slides and stained with Wright's stain. Wintrobe's Hematocrite was employed for the determination of packed red cell volume.

(b) *Biochemical techniques.*—Plasma iron was determined by a modified technique of Hamilton (1950). To 1.5 c.c. of plasma was added 0.75 c.c. of 2 N hydrochloric acid and the mixture allowed to stand for 10 to 15 minutes after thorough shaking. 0.75 c.c. of trichloroacetic acid was then added with vigorous stirring with a glass rod. This mixture was left for 20 to 30 minutes, and then centrifuged at 3,000

r.p.m. for 30 minutes. The supernatant was decanted off. To 1.8 c.c. aliquot of the supernatant were added the following in this order :

0.05 c.c. thioglycolic acid.

0.40 c.c. (0.1 g./100 c.c.) O—phenonthroline.

0.75 c.c. saturated sodium acetate.

The tube was shaken and allowed to remain at room temperature with a reagent blank (0.90 c.c. glass distilled water, 0.45 ml 2N HCl and 0.45 ml trichlor-acetic acid *plus* reagents above) for 30 minutes. Optical density was measured in a Beckman's spectrophotometer model B at 510 m μ .

Total and unsaturated iron-binding capacity as also the per cent saturation of iron in the plasma were calculated as suggested by Cartwright and Wintrobe (1949). For the determination of plasma copper and the copper content of the red blood cells and the whole blood, the techniques of Gubler *et al.* (1952) were followed.

Glassware used in the collection, storage and estimation for iron and copper was carefully cleaned to make it copper-iron free by washing thoroughly with soap and water, rinsing, immersing in potent potassium dichromate-sulphuric acid cleaning solution for one hour, and then rinsing with tap water, simple distilled water, and finally with double-distilled water, prepared in all-glass still. All the reagents used, including anti-coagulants were of A—R grade and of known iron-copper content. Matching of colour was performed on a Beckman spectrophotometer model B at 510 m μ for iron and 440 m μ for copper.

Determinations on plasma proteins (micro-kjeldahl method), alkaline phosphatase, phosphorus, calcium and blood sugar have been carried out according to King (1956).

Results.—The normal range of various determinations obtained on healthy periodontal individuals are shown in the following table.

Determination.	Number Investigated.	Range.	Mean value.
Red cell count $\times 10^6$ /cu. mm.	55	4.10— 6.00	4.90
Haemoglobin, g. per cent	75	12.00— 18.00	14.70
Packed cell volume, per cent	75	38.00— 50.00	43.00
Plasma iron, mg. per cent	75	70.00—160.00	116.00
Plasma copper	55	95.00—180.00	145.00
Serum proteins, g. per cent	75	8.28— 5.90	7.27
Albumin, g. per cent	75	3.60— 5.70	4.46
Globulin, g. per cent	75	1.30— 2.49	2.71
Alkaline phosphatase, K.A. units	75	4.50— 14.00	8.50
Serum phosphorus, mg. per cent	20	1.40— 4.30	2.90
Serum calcium, mg. per cent	20	8.50— 10.70	9.50
Blood sugar, mg. per cent	20	70.00—109.00	90.00

- 130. Investigation to gauge the value of various endodontal treatment for the abscessed pulpless deciduous and young permanent teeth under Dr. Vimla Sud at the Safdarjung Hospital, New Delhi.**

The main objects of inquiry are to find out whether it is possible to retain the abscessed deciduous and young permanent teeth by instituting a suitable endodontal treatment so as to avoid the complications which result from their early loss. The most suitable endodontal procedure for this purpose is being determined by injecting 4 types of materials, in the root canals and observing progress of the healing changes in the bone and supporting structures. The materials used are : (i) Mynol, (ii) Zinc oxide-eugenol with a drop of formocresote. (iii) P.B.S.C. paste, and ammoniacal silver nitrate.

The material for the inquiry was obtained by survey of schools during which the children suffering from abscessed pulpless teeth were selected and subjected to a thorough examination according to proforma specifically prepared for the purpose. The affected teeth were x-rayed and the existing changes in and around them carefully assessed. The children were then divided into 4 groups on the basis of the material injected. Healing changes were then carefully followed by clinical and radiological assessment, first at monthly interval and then bimonthly. The material producing better and quickly healing will ultimately be recommended for use and details of procedure devised in the light of the experience gathered will be furnished.

So far 787 children have been surveyed and out of them 54 have been found to be suffering from abscessed teeth. Twenty out of them have been given the above treatment 10 with mynol and 10 with zinc oxide eugenol with a drop of formocresote. Their follow-up is proceeding.

ENVIRONMENTAL HYGIENE & SANITATION

- 131. Industrial wastes disposal and water pollution research unit at the All-India Institute of Hygiene and Public Health, Calcutta.**

Proper sanitary disposal of liquid wastes, particularly industrial wastes, has proved a difficult problem in different parts of the country. The water pollution problems, arising out of the wastes, have also been of concern to the public health authorities in certain regions. One of the urgent problems facing the health authorities in the country is proper disposal of industrial wastes in the Uttar Pradesh and Bihar States. In view of the foregoing considerations, the Industrial Waste Disposal and Water Pollution Research Unit was established at the All-India Institute of Hygiene and Public Health, Calcutta. Field Units in the States of Uttar Pradesh and Bihar were also established to carry out

comprehensive survey of the wastes and investigate water pollution problems arising out of them.

The Unit has continued research work on treatment of sugar and distillery wastes during the year. Laboratory and pilot plant studies have been carried out with a view to find out suitable methods of treatment and disposal of these wastes. Although no satisfactory method of treatment of these wastes has yet been worked out, the results obtained so far have shown the limitations of some of the conventional methods and also indicated the lines of further work that may lead to practical results for treatment of the wastes. As a result of these studies it has been found that some species of tropical algæ are of value in purifying partially treated distillery waste. In this connection 36 specimens of green algæ were studied. It was observed that two species, *Hydrodictyon* and *Chlorella* were useful for the purpose. An apparatus for study of oxygen donating power of different algæ under controlled conditions was fabricated and is being used for these studies.

Quantitative experiments on the use of these two species have shown that ponding of distillery wastes inoculated with the cultures of these species of algæ bring about reduction in B.O.D. and colour of the waste.

The results show that with 1:40 dilution of distillery waste the *Hydrodictyon* produces an effluent with a B.O.D. of 2.5 to 10 ppm and *Chlorella* with a BOD of 2.5 to 15 ppm.

The results obtained on trickling filter treatment of distillery waste have shown that this method of treatment of spent wash may not be economical because the efficiency of purification is low. A laboratory model Bio-filter, in which the recirculation factor can be easily manipulated has been built for the study of the efficiency of this method of treatment in handling distillery and sugar wastes. It was also observed during the survey of industrial wastes in Uttar Pradesh that one of the promising lines of work in regard to disposal of distillery wastes, is separation of the spent wash and its concentration and burning to yield potassium salts. A pilot plant for this purpose has been designed in consultation with one of the distilleries in Lucknow. This method of treatment of distillery waste will be useful particularly in areas where there is no large water course or sufficient land available for its disposal without creating nuisance. The method will also lead to recovery of valuable by-product in the form of potassium salts, which are in short supply within the country.

The Unit in cooperation with the Indian Lac Research Institute, Ranchi, has designed a common treatment plant for proper treatment and disposal of lac wastes produced in Tulin village, where a population of about 4,000 are engaged in the manufacture of seed lac as a cottage industry. The treatment plant, costing about Rs. 1.5 lakhs, when installed will go a long way in mitigating insanitary conditions in which the villagers live in this region.

A pilot plant for treatment of sugar waste was installed and operated at Bihta Sugar Factory, Bihar. Observations on the pilot plant have been carried out during 1956-57 sugar season. The results show that (a) 60 to 70 per cent of the organic load of the waste is removed by digestion for 3 days (b) the efficiency of purification of digested waste on the trickling filter is rather low. Based on these observations, the pilot plant has been modified to provide digestion of waste in stages for 6 days followed by chemical treatment or ponding with algæ. Observations on this pilot plant will be carried out during the 1957-58 sugar season.

During this period field units have been established in the States of Bihar and Uttar Pradesh. The staff of the Unit are now well trained to carry out waste survey and related water pollution investigations. Laboratories have also been organised in the Provincial Hygiene Institute at Lucknow for U.P. and the Public Health Institute at Patna for Bihar, in order to carry out analyses of wastes and other related work of the Units. The Units work in close collaboration with the Health and Public Health Engineering Departments of the States.

During this period the Bihar field unit has carried out detailed survey of the wastes in Zone I and part of Zone II. The results of the survey showed that Zone I is mainly agricultural with some concentration of industries in Dalmianagar. It was reported that wastes from the Dalmianagar Group of Industries create water pollution in the Sone River during the summer months. The survey showed that majority of the sugar factories and distilleries are located in Zone II. Satisfactory disposal of wastes from these factories presents a difficult problem in this region. The factories are located in areas where there are no big river or sufficient land available to receive the wastes without creating nuisance in the locality. Until simple and cheap methods of treatment and disposal of sugar waste are worked out, it is difficult to suggest a satisfactory solution to this problem.

The sources of water pollution in Zones I and II have also been located. It is proposed to organise river survey work during 1958-59 with a view to determine the degree of treatment necessary in the individual cases for mitigation of water pollution in this region.

The field unit in Uttar Pradesh has carried out survey of sugar, distillery, tannery and a few textile mills in the State. The survey has shown that apart from sugar and distillery wastes, one of the urgent waste disposal problems of the State relates to tannery wastes. A comprehensive survey of this problem has been made with a view to suggest remedial measures. Arising out of this survey, a proposal has been made for the proper disposal of tannery wastes. The proposal embodies the following recommendations—(i) No tannery be permitted to discharge its wastes directly into the river without treatment; (ii) All tanneries provide properly covered drains to carry wastes into the sewers; (iii) All tanneries construct fill and draw sedimentation tanks as per specifications approved by the Public Health Engineering Department of the State for the removal of suspended solids before the wastes are discharged into the river or municipal sewer and (iv) the flow of the tannery effluents should be regulated so as to ensure proper dilution with sewage at all times.

In co-operation with the State Governments, Regional Committees have been formed in each of the States to review the work of the field units and to coordinate the activities with similar work in the States. The Regional Committees had their first meeting in December, 1956.

132. Enquiry into the effect of sewage treatment on tubercle bacilli at the All-India Institute of Hygiene and Public Health, Calcutta.

The presence of tubercle bacilli in sanatoria sewage, effluents and sludge has been demonstrated by workers, in different parts of the world. It is possible that release of sewage effluents, and sludges from sanatoria and waste water from slaughter houses without proper treatment may constitute a public health hazard. It is necessary, therefore, that satisfactory methods of treatment of these wastes are prescribed if risks arising out of improper disposal of sanatoria wastes are to be mitigated. Work on this problem was, therefore, undertaken with a view to determine the efficiency of removal of tubercle bacilli in the existing sewage treatment plants in sanatoria and to work out procedures for proper treatment of effluents and sludges before they are disposed off on land or water.

Observations have been carried out in two sewage treatment plants in the Jadavpur and Kanchrapara sanatoria near Calcutta. In Jadavpur the sewage from the sanatoria is collected in a sump and treated in a biofilter. The effluent is let into a jill from where it overflows through drain on the adjoining land.

In Kanchrapara, the sewage from different sections of the sanatoria are treated in a number of septic tanks from where the effluent is let on a stone bed before it passes into the open Nala leading to the canal.

Samples of sewage at different stages of treatment (raw, sedimented effluent and sludges) have been collected periodically and examined in the laboratory. Forty-nine sets of samples have been examined from the Kanchrapara plant and 36 from Jadavpur. These samples have been examined microscopically and also cultured for isolation of tubercle bacilli using a standardized technique evolved for the purpose. Most of the samples examined so far, including the final effluents from the treatment plants showed the presence of acid fast bacilli by microscopic examination. Cultures were also positive in a large percentage of the samples examined. The results obtained are summarized below :—

Nature of Sewage	Jadavpur		Kanchrapara	
	Number of samples	Percentage positive	Number of samples	Percentage positive
Raw	36	95	49	94
Sedimented	19	84	37	90
Final effluent	18	72	26	85
Sludge	3	100	3	67

It was also observed that the number of colonies developing on media inoculated with effluent samples were much less as compared with the raw and sedimented sewage samples.

The samples collected from the treatment plants were also analysed for suspended solids and B.O.D. in order to determine the efficiency of purification of sewage obtaining in the treatment plants. The results obtained showed that the efficiency of purification as judged by B.O.D. removal was 80 to 95 per cent in the Jadavpur plants. The suspended solids results were some what erratic because of septic condition and sludge escaping in primary effluent. The results obtained from chemical analysis of the Kanchrapara samples were not consistent and indicated low efficiency of purification as judged by B.O.D. and suspended solids.

It was also observed during these studies that the Jadavpur plant went out of order quite often due to mechanical defects and remained idle for a good part of the year, when raw sewage was directly discharged into the jill. It was also observed that the arrangements for treatment of sewage in the Kanchrapara plant are not satisfactory. The sewage is let into a number of septic tanks which overflow and flood the neighbouring area creating insanitary conditions. Pools of sewages stagnates in the area during the rainy season and the condition of the locality is appalling.

The viability of T.B. cultures isolated from the different samples were also tested by animal inoculation experiments.

Both cultures and processed sewage samples were used for inoculation. Six lots of animals including three lots of albino guinea-pigs from the Guindy Institute have been inoculated. Some of the guinea-pigs in the first lot died of septicæmia in spite of penicilin treatment within a few days. Although all the inoculated animals did not show symptoms of disease a few of them which died within 2 to 3 weeks showed definite lesions and acid-fast organisms. It was also observed that those which survived recorded considerable loss in weight during this period. The albino guinea-pigs which have recently inoculated are still alive and under observation. Some of them have recorded loss in weight. They are still alive and are under observation.

Experiments have also been carried out with a view to determine the satisfactory procedure for chlorination of effluents to destroy the T.B. bacilli. These experiments have been carried out both with effluents obtained for treatment plants as well as suspension of T.B. germs prepared in the laboratory. These suspensions have been exposed to varying doses of chlorine for different periods. The results so far obtained indicate that a dose of chlorine sufficient to leave a residue of 1 to 3 p.p.m. and contact time of 30 to 60 minutes is necessary for efficient disinfection of the effluent.

133. Study of maintenance of small tube wells in rural areas of Bihar under Sri K.N. Rohatgi, P.H. Engineering Department, Bihar, Patna.

Collection of data, after day to day observation of the life of hand pumps, their component parts, life of strainers, sub-soil water level, yield of tube wells in different periods of the year, and periodical chemical analysis of water of tube wells has been made. Realizing the vital role played by the human elements involved in the proper maintenance of tube wells, a count of literate persons in each village has also been made. Out of 290 tube wells under observation 24, which were found to be choked up during the period, were taken out and resunk. Owing to lack of laboratory facilities water of tube wells only was chemically analysed during the period.

The day to day observation is necessary for a longer period in order to arrive at some definite conclusions regarding the maintenance of small tube wells and their hand pumps.

134. Study on the different types of latrines used in rural-areas under Professor N. Majumder at the All-India Institute of Hygiene and Public Health, Calcutta.

Drawings and moulds of various types of squatting plates in rural areas of India have been collected at this Centre. So far, the observations indicate that the design followed by Poonamalee Health Centre is perhaps the most effective one with respect to flushing of excreta through the water seal.

The unit in Punjab started functioning on August 20, 1957. Information relating to different types of latrines used in rural areas of this region is being collected.

The fourth unit has not yet been established.

Conclusion.—Government permission for the establishment of the units in the different states is taking considerable time. It has not been possible to obtain the full complement of the staff in any one of these units. The appointment of Co-ordinating Officer has been made only recently. It is hoped that the progress of these units will be more satisfactory and conclusive data may be obtained in a couple of years.

INDUSTRIAL HEALTH

135. Industrial Health Research Unit under Dr. M.N. Rao at the All-India Institute of Hygiene & Public Health, Calcutta.

A. INDUSTRIAL HYGIENE :

Air pollution.—The study on the nature of air pollution in Calcutta is continuing. The average monthly dust fall per square mile area of Calcutta has been found to be 53.63 tons per square mile.

The analysis of the air samples for gaseous concentration has continued during the year and the results are shown below :—

	Conc. in parts per million.	
	1955-56	1956-57
Sulphur dioxide	0.013-0.076	0.023-0.064
Oxides of nitrogen (NO ₂)	0.040-0.150	0.029-0.091
Ammonia	0.143-0.593	0.152-0.355
Aldehyde (as formaldehyde)	0.070-0.300	0.050-0.150

A new item of this year's study in air pollution is the determination of air-borne dust in the atmosphere. The amount of dust floating in the air was found to vary between 0.014 and 0.563 mg. per cubic meter of air.

A study of the diurnal variations of air pollutants showed that though individually the concentrations are different for different substances, their concentrations were uniformly high in the morning and evening and a drop during mid day.

B. INDUSTRIAL HEALTH-PHYSICS.

1. *Film badge dosimetry.*—With the object of standardizing a monitory service suitable to small installations in India, a tentative experiment (weekly) was conducted to find out the minimum period for which the same film badge may be used to estimate the limits of accuracy. Cheap film holders fabricated in the Unit's workshop are being used for monitoring the technicians exposed to x-ray radiation in nearby hospitals.

2. *Radiation hazard in non-medical installations.*—(a) Radiography in industry is commonly used for detecting defects in the finished products of engineering industries. A group of 18 installations are being contacted and radiological load data collected.

(b) Health hazards in radiation work in a radiation laboratory in Calcutta where radio-active materials are handled, are being studied.

3. *Physical aspects of heat regulation.*—Using the conductometric technique for estimation of salt concentration and using the specially designed bicycle ergometer, a series of experiments are being conducted for the continued study of the physiology of exercise. Eleven subjects are being tested in an improvised psychrometric chamber in different seasons.

4. *Voluntary respiratory arrest time.*—This respiratory function test during rest and exercise is being studied to ascertain whether this can be utilized in the physical fitness standards.

5. *Workshop activities.*—The workshop which in the past had been utilized for designing important laboratory equipment, e.g., pedalling bicycle ergometer, etc., has been improved with equipment and staff loaned by this Institute.

C. INDUSTRIAL PSYCHOLOGY :

1. *Study on industrial tension—further analysis of the results.*—The preliminary results of the action-research programme which included orientation programmes for both the supervisors and the workers in an engineering industry near Calcutta were reported last year. Further analysis of the results show the influence of other variables on workers' morale and the following conclusions are drawn :

(a) Education shows an inverse relation with high morale of the workers. One of the reasons of dissatisfaction among the educated personnel was that they found difficulty in adjusting to the industrial situation. The necessity for a scientific induction system for the workers in a new industrial environment is, therefore, emphasized.

(b) Workers' monthly pay is also found to be an influencing factor related to workers' morale.

In general, no significant correlation between the scores in the individual factors comprising the attitude test scale was observed. But the correlations between the morale scores and the scores in each of the said individual factors were found positive and significant.

2. *Study on fatigue in industry.*—A pilot project for assessing the problem before undertaking a full-scale research project on fatigue and workers' efficiency has been conducted by the Unit in the "Capstan Production Line" of a leading engineering industry near Calcutta. The work of this project has recently been extended to a second factory where 6 workers were studied in all the 3 shifts for 3 weeks at a stretch. No significant differences were seen between production rates of individual workers in different shifts. A constant trend in increased hourly production, especially during the early post-lunch period, was seen.

D. SOCIAL SCIENCE :

The medico-social worker and the health visitor of the Unit are completing a survey of the health status of industrial workers in the Urban Health Centre, Chetla. This survey of workers in unregistered small-scale industries includes their occupational and home environment.

E. APPLIED PHYSIOLOGY :

The work of the physiology branch of the Unit was restricted due to the Assistant Research Officer being in Germany since October 1956 on a fellowship.

The work on differential lung volumes is being published during the year.

F. INDUSTRIAL MEDICINE :

Excretion of coproporphyrin in health and disease :

Quantitative urinalysis for coproporphyrin in normals and some occupational groups allied to workers exposed to lead continued.

G. SERVICE TO INDUSTRY.

The pressure on the Unit for technical advice and service is increasing since the publication of the popular brochure on the Industrial Health Research Unit.

The following are some examples :—

- (a) A study in the working conditions of a Post and Telegraph Workshop :

A clinical environmental and biochemical investigation into complaints from the workers in the galvanising section of the workshop was conducted. An industrial air analysis for contaminants like zinc, lead and ammonia and urinary estimations for coproporphyrin showed no abnormal exposures.

- (b) Heat Load Problem in a Foundry :

One engineering industry in Calcutta requested the Unit to study the heat load in its foundry. A study was made to find out the heat load in summer. High effective temperatures (beyond 90°) were prevalent in a number of areas. The various sources and factors contributing to the thermal discomfort were studied and a recommendation for improvement of the environment was made to the industry.

136. A study into incidence of pneumoconiosis in Central Indian Coalmines under Dr. K.B. Roy, Medical Officer, Nowrozabad Colliery, Nowrozabad.

A total of 259 underground workers belonging to the category as defined in Technical Report (1956) were examined clinically and radiologically. Among these, 20 patients were suffering from complicated pneumoconiosis, 21 patients from simple pneumoconiosis and the rest were negative.

All patients with complicated pneumoconiosis except one have worked in underground jobs for more than 20 years ; eight of them have been disabled by their pulmonary condition so much so as to be compelled to give up their job as minor. Findings in respect of blood ESR were similar to that mentioned in the last report.

Coalminer's pneumoconiosis is a fairly prevalent disease among senior coalminers in Central Indian coalfields.

137. Inquiry into absenteeism under Dr. H.P. Dastur, Medical Officer, Tata Industries (Private) Ltd., Bombay.

This is the second interim report of the inquiry on absenteeism based on the data obtained in the Spinning and Carding Departments of Tata Mills, Dadar, Bombay.

Progress of work.—Work continued on the same lines as the previous year from July 1956 to June 1957 with 678 workers of the Spinning Department and from September 1956 to September 1957 with 421 workers of the Carding Department.

All interviewing work was postponed from October 1, 1957, in order to analyse the data. As the time was short, it has so far been possible to analyse the data of 669 absentees of the Spinning Department. To make sure that the analysis be as statistically correct as possible, the Department of Economics and Statistics, Tata Industries, was consulted and with their help the research workers have tabulated all data on a special form. To further facilitate the tabulation of the data 669 absentees were divided into 29 more or less homogenous groups, for instance according to shift, sex, occupation and so on.

Factors selected for analysis were :—

- (1) Rate of general absenteeism.
- (2) Causes of absenteeism and the man-days lost per cause.
- (3) Frequency of absence and its nature.
- (4) Absence in relation to age.
- (5) Sickness absenteeism.

Analysis have been made on following counts :—

- (1) General absenteeism.
- (2) Causes of Absenteeism.
- (3) Frequency of absence.
- (4) Correlation of absence with the pay-day, holiday and authorised leave,
- (5) Absence in relation to age.
- (6) Rate of sickness absenteeism.
- (7) Breakdown by causes of uncertified absence.
- (8) Breakdown of sickness certified by panel doctors of the Employees State Insurance Scheme.
- (9) Sickness absence according to duration.

138. Study on bagassosis at the Vallabhbhai Patel Chest Institute, Delhi.

A co-operative research programme, including both the experimental and clinical aspects, was undertaken at the Vallabhbhai Patel Chest Institute, Delhi University, Delhi. The clinical studies were made at the Bagasse Plant of the Rohtas Industries factory in Dalmianagar,

Bihar, as a case report of this disease is known to have appeared from there.

The experimental work done in our laboratories included the biochemical study of the organic and inorganic fractions of bagasse fibre, bacteriological and mycologic flora of the dust and pathologic effects of induction of this condition in experimental animals. A brief summary of the work done and results obtained is given below :—

In the biochemical studies, bagasse from different parts of India has been examined for its protein, fat, carbohydrate, crude fibre, total silica, calcium and phosphorus content.

In the bacteriological studies, a special medium containing bagasse extract was prepared, and the bagasse fibres, after having been washed repeatedly with sterile distilled water were cultured on nutrient agar and this medium. All the organisms isolated were sporebearing bacilli, with minor differences in biochemical characteristics. No single organism was found to be predominant in the several samples examined. Cultural examination of the sputa of workers exposed to the dust in the bagasse plant did not yield significant information.

The fungi so far isolated out of the bagasse fibers include *Aspergillus niger*, *Penicillium species*, *Mucor sp.*, and *Rhizopus species*, etc. These are mostly the same as were present in the laboratory atmosphere of the Institute. *Aspergillus fumigatus* and *Helminthosporium species* were, however, isolated only from Bagasse and as the former has been incriminated by some workers as an important organism in the etiology of bagassosis, it was used for experimental studies on animals.

The experimental pathology studies on animals were done in 2 parts. (1) Acute changes produced by 1—2 exposures to bagasse. (2) Chronic changes produced by prolonged exposure to the dust in the dust chamber and factory itself. The acute changes were induced by exposing the animals to 200 mesh dust in an improvised plastic mask for a few minutes and also by giving 2 injections of a 2 per cent suspension of the dust into the trachea at an interval of 2 days. Changes induced by the latter method were more severe and diffuse and observed upto 130 days after the last exposure. They consisted of a severe degree of thickening of the alveolar septa owing to marked swelling of the septal cells and congestion of alveolar capillaries. In places the thickening was so severe, as to obliterate the alveolar lumina. In addition, there were mononuclear infiltrates, but the changes in guinea pigs were almost exclusively interstitial. Reaction in cats was somewhat different and consisted of lobular pneumonia, with polymorphonuclear response. No tendency to organization was observed upto 130 days in this group. The reaction was not interpreted to be due chiefly to be due to the foreign particles induced. Animals treated with autoclaved bagasse showed changes which were indistinguishable both in extent and type, to those induced by native bagasse. Cultures of bacterial or fungal organisms when introduced in equivalent amounts showed profound pneumonic changes in addition to the generalized interstitial reaction. The protein, carbohydrate extracts and total inorganic ash

suspension were also used in similar experiments. Only the carbohydrate fraction was observed to induce changes somewhat similar to those produced by native bagasse, but the results are not conclusive.

In the second part of the study, rabbits and guinea-pigs were exposed in dust chamber, improvised by us in our own workshop, and also in the actual working conditions of the factory. A batch of animals were kept in individual cages, at 4 different locations in the bagasse plant of the factory. The sites were chosen according to the gradation of dust concentration, as determined by the concentration studies. The dust concentration in the laboratory dust chamber was roughly equivalent to that in the factory. In the dust chamber the animals were studied after a total 2 to 70 hours exposure. In the factory, the animals were exposed for 8 hours per day and sacrificed after a total 72 to 128 hours of exposure. Changes induced by the above 2 methods were similar and were observed even 4 hours after exposure. In these animals, a combined alveolar and interstitial reaction was observed, and it also included many polymorphs. In the rabbits, it was predominantly polymorphonuclear. No normal lung tissue was seen after 12 hours exposure. Some degree of organization was observed in 2 guinea-pigs exposed in the dust chamber over a period of 48 hours. The most significant point in this study was the fact that the animals which were exposed in the factory for 1 to 2 weeks at location where the concentration was minimal, showed appreciable pathologic changes in the lungs indicating the seriousness and magnitude of the clinical problem.

In a clinic survey of this problem, 105 workers exposed to the bagasse for more than 60 days dust were examined. The examination consisted of complete physical, fluroscopy, röntgenography, hematology examination, exercise-tolerance tests and antigen tests. Out of these, there were only 55 workers who were exposed purely to bagasse dust and the remaining were also exposed to other toxic gases in addition. Most of these patients had respiratory symptoms and 18.5 per cent had history of hemoptysis. No characteristic picture was found on x-ray, hematologic examination and antigen tests were also non contributory. A practical difficulty faced was that the workers employed in the bagasse plant, work on a daily wage basis and most of them do not work consistently over a period of time. They leave either when symptoms get more severe or when the harvest season is on and therefore a proper follow up was not possible.

139. Nutrition studies in relation to environment under the Director, Nutrition Research Laboratories, Coonoor.

Industrial nutrition has not received in India the attention it deserves. A closer and detailed study of nutritional problems of industrial labour has become necessary in view of the rapid industrialization of the country within recent years and the probability of further industrial expansion.

A research project on nutrition in relation to factory environment in textile industry has been undertaken with the advice and help of the

Industrial Health Advisory Committee of the Indian Council of Medical Research. During the year under report two Units—one in Ahmedabad and one in Coimbatore—have started functioning. Diet and nutrition surveys of the workers and their families have been undertaken as a preliminary stage in the investigation. Nutrition surveys have so far been completed on 400 workers in Ahmedabad and 700 workers in Coimbatore. Diet and socio-economic surveys have been carried out in about 100 families in each area. Further work is in progress.

M A T E R N A L & C H I L D H E A L T H

140. Hæmatological investigations of the new-born babies of anæmic mothers under Dr. R.K. Dutta Chaudhuri at the Chittaranjan Seva Sadan and Cancer Hospital, Calcutta.

M A T E R I A L A N D M E T H O D S

538 cases have been studied this year, which included 197 mothers, 211 new-born babies; 100 follow-up infants (upto 2 years of age) and cross sectional study of 30 infants.

Hæmatological study of 26 newborn babies of normal mothers and 90 newborn babies of anæmic mothers (normocytic hypochromic—22, microcytic hypochromic—25, macrocytic—18 and dimorphic—25) were done.

Serum iron values of 20 normal and 23 anæmic mothers alongwith their babies have been studied.

Serum iron values of 10 cord bloods were also included. Serum proteins (different fractions) have been studied in 20 normal and 18 anæmic mothers and their respective babies including cord blood in 12 cases were also the subject of study.

Methods employed were same as described in the Technical Report of the Scientific Advisory Board for the year 1956.

O B S E R V A T I O N S A N D S H O R T C O M M E N T S

1. No significant difference between the hæmatological values of babies of normal mothers and those of the anæmic mothers was observed.

2. Hæmatological values of the premature babies of normal mothers also did not differ significantly from those of anæmic mothers, but the average hæmoglobin values of premature babies were higher than those of the mature ones irrespective of the hæmatological status of the mother.

3. Simultaneous bone marrow smear study of mothers and their babies (3 normal and 11 severely anæmic mothers) indicated correlation-ship between them as regards the type of anæmia the mother suffered from.

4. *Findings of the follow up babies of normal and anæmic mothers.*—From the study of 100 mature babies (28 of normal mothers of 72 of anæmic mothers) which were subjected to periodical investigations at various age periods (only 8 could be followed up to 2 years of age), it seems that the babies of anæmic mothers developed anæmia from 6 months onwards (8.03 g. per cent on average). Their average hæmoglobin came down to 7.65 g. per cent at 9 months age and they remained anæmic even up to 2 years of age though their hæmoglobin value went up to 9.6 g. per cent.

The premature babies of both normal and anæmic mothers developed anæmia from third month onwards (8.68 g. per cent on average) and became severe in late infancy when left untreated. To some extent the premature babies of anæmic mothers remained more anæmic as compared to those of normal mothers the average values being 8.88 g. per cent in those of normal mothers and 6.8 g. per cent in babies of anæmic mothers.

5. Average hæmatological values of normal infants up to 2 years age show that mean corpuscular volume and mean corpuscular hæmoglobin decrease as the child grows.

6. *Serum iron studies on mothers and their babies:* Serum iron values of 20 normal mothers and 29 anæmic mothers, 15 babies of normal mothers and 21 babies of anæmic mothers have been studied.

Iron values of cord blood were not influenced by low serum iron of the mother.

7. *Findings of different fractions of serum protein of mothers, mature babies and premature babies:* Different fractions of serum protein of 20 normal mothers and their babies; 18 anæmic mothers, 14 babies of anæmic mothers and 10 premature babies were done. The results showed that there was no significant difference between total serum protein values of babies (4.8 to 6.4 g. per cent) of normal mothers and those (4.4 to 6.6 g. per cent) of anæmic mothers.

Total serum protein as well as the different fraction of the protein of the premature babies at birth were lower than those of the mature ones.

141. Study of the nutritional status of expectant mothers with particular reference to the incidence of toxæmias of pregnancy and anæmias in pregnancy in Northern Madhya Bharat, under Dr. (Miss) L.V. Phatak at the G.R. Medical College, Gwalior.

Normal-looking pregnant women at the stage of first trimester of pregnancy were taken up for this work. Some have been admitted for study in the second trimester. They had to fulfil the following requirements for starting investigations :—

1. No signs of anæmia and toxæmia should be present.
2. They must be able to attend the hospital throughout pregnancy and have the labour in the hospital.

3. The patients have to be residents within the municipal limits to make the work of the Health Visitor possible regarding the dietetic study.
4. Recently, a study of the diets in established cases of toxæmia and anæmia admitted to the wards has been started.

I. The patients are examined in a special clinic, after selection from the general out-patients. At the clinic, besides the entrance of details in the proforma used, a routine antenatal check up is done by a thorough physical examination of the patient and blood pressure reading is taken in addition to the urine examination for the presence of albumin.

II. From here onwards the patients attend the clinic in the second and third trimesters of pregnancy for a similar examination, or in any other week for other treatment.

III. After labour the infants born to these women have been studied for their weights, wherever possible. The findings have been correlated with the nutritional status and the complication of anæmia, toxæmia and hypertension that the mothers might have developed later.

IV. It is difficult to find patients attending antenatal clinic in the first trimester excepting when there has been a bad obstetric history. The result is that, fewer patients than the number registered can be followed to term as a result of abortion or premature labour in these women. Besides this, an insistence on a longitudinal study numerically reduces the numbers available.

V. Therefore, along with the patients whose studies began in the first trimester, some that presented themselves in the second trimester and were agreeable to the above-mentioned conditions have also been included in the study.

VI. The per capita income was verified in 267 cases and the patients divided into 4 groups. Out of the 280 cases under consideration, diets have been studied in a total of 274 cases. The remaining 6 patients have been normal in their pregnancies. The diet values of the food consumed have been assessed according to Health Bulletin No. 23 of the Research Laboratories, Coonoor.

The other factors of diet, i.e. fats and carbohydrates, are not considered vital in their amount of intake and the minimum standards are not yet calculated for pregnancy.

A total of 280 cases have been studied and out of these 141 have had normal pregnancies and labour without any manifestation of anæmia, toxæmia or hypertension. Seven cases have had premature labours and 6 cases have had an abortion since their enrolment for this study. Ninety one cases are being followed in different trimesters of pregnancy while 35 remain untraced.

The analysis of the types of anæmias in the 23 cases shows the following figures :—

(N.N.) Normocytic normochromic	...	11
(N.H.) Normocytic hypochromic	...	3
(M.H.) Microcytic hypochromic	...	8
(Ma.H.) Macrocytic hypochromic	...	1
		23

Hypertension.— Nine cases manifested only raised blood pressure, 3 in the second and third trimesters and 6 only in third trimester. All these showed no complication of pregnancy and settled to a normal blood pressure within 10 days of delivery.

Evaluation of the weights of the new born.—The average weight of a baby born in this province has been determined as 6.132 pounds. All the babies born to mothers with either of the three complications mentioned above have been weighed.

The average weight works out to be for—

Anæmia	...	5.34 pounds.
Toxæmia	...	5.55 pounds.
Hypertension	...	5.91 pounds.

142. Inquiry on the nutritional status of expectant mothers, with particular reference to the incidence of toxæmias of pregnancy and anæmias of pregnancy under Dr. V.S. Mangalik at the King George's Medical College, Lucknow.

This inquiry includes a study of normal pregnant women between 20 and 28 weeks of pregnancy, selected from the general ante-natal attendance, who were followed till term to observe the incidence of toxæmia and anæmia. As the number of such cases was meagre, therefore, women who were admitted in the hospital with the diagnosis of anæmia or toxæmia were also included.

In this study, the nutritional status of pregnant women was assessed on the basis of clinical examination in order to determine the various deficiencies, the rating of diet, and certain biochemical and hæmatological investigations. The biochemical studies carried out, included the estimation of total proteins chemically and their various fractions electrophoretically, and determination of serum calcium and phosphorus. Hæmatologic studies included determination of hæmoglobin and volume of packed red cells. RBC counts and sternal smear examination were carried out when indicated.

Total number of cases studied were 389 pregnant women. Out of these, 300 were those who had been registered in the ante-natal clinic,

of which only 105 could be followed till term. Of the remaining 89 patients studied, there were 39 cases of toxæmia and 50 of anæmia.

Observations.—A study of the income levels in these women showed that no significant relationship could be established between the income level and the incidence of toxæmia. However, of the anæmic subjects, 75 per cent belonged to an income group of below Rs. 150 monthly.

Maternal weight studies showed that toxæmic subjects were usually over weight, whereas most anæmic women were underweight.

The clinical findings of calcium and vitamin deficiencies were usually notable, particularly in the eyes, oral mucosa, teeth, bones and skin. The commonest deficiency manifestations during pregnancy were stomatitis and glossitis. The next in the order of occurrence were bleeding gums and carious teeth. Other deficiency manifestations, such as drying of skin, conjunctival and corneal xerosis, paresthesia and calf tenderness were less common. The occurrence of these signs and symptoms in toxæmic women was more or less the same as in normal pregnant women, whereas in cases of anæmia the incidence was found to be greater.

Pregnant women were usually undernourished and many of them were consuming diet that could be categorized as 'poor' or 'very poor.' The nutrients commonly deficient in the diet were proteins, calcium, vitamin A, riboflavin and ascorbic acid. The pattern of dietary intake of toxæmic pregnant women did not differ significantly from that of normal pregnant women. The anæmic subjects were found to be usually more undernourished and the deficiency of various nutrients was more pronounced.

Analysis of serum proteins in normal pregnancy showed that there was a tendency for the serum proteins to fall as pregnancy advanced and the drop was more marked in the albumin fraction, as evinced by a decrease in the A.G. ratio. In cases of toxæmia, the lowering of total proteins was even greater and again due to a decrease in albumin. Electrophoretic analysis confirmed the above findings and further revealed a change in the various globulin fractions. Alpha and beta fractions showed some increase, while the gamma fraction did not fluctuate much. No significant relationship was found between the dietetic intake and serum protein levels.

Serum-calcium estimations revealed very insignificant changes in cases suffering from toxæmia and anæmia. Similarly, the values of serum phosphorus in toxæmia and normal pregnant women were alike.

Hæmatologic analysis of the toxæmia cases revealed an associated anæmia in 12 cases, out of which 9 showed normocytic normochromic blood picture. In the other 3, the anæmia was macrocytic. In none of these was the bone marrow megaloblastic.

143. Inquiry on the nutritional status of expectant mothers under Dr. P.K. Devi at the Medical College, Nagpur.

Five groups of cases were examined with a view to assess the nutritional status according to the schedule circulated. *Group A*—It consisted of 130 cases attending the antenatal clinic, out of these 100 cases have delivered. *Group B*—Consisted of 105 cases of anæmias of pregnancy admitted in the wards with less than 8 g. of Hb. per 100 cc. Thirty per cent of these cases had associated toxæmia also. *Group C*—70 cases of toxæmia admitted in the wards were included in this group. *Group D*—consisted of 100 non-pregnant women attending the Gynæcological section of the Out-patient Department. Xerosis of the conjunctiva with or without Bitot's spots, Spongy bleeding gums, and anæmia constituted the commonest types of disorders seen. They were all seen least frequently in the non-pregnant group showing that pregnancy is an important conditioning factor. *Group E*—consisted of 231 cases of pregnancy in all trimesters. On analysing the incidence of deficiency syndromes in these cases according to the duration of pregnancy, the incidence of Vitamins A, B and C deficiencies and anæmias increased progressively with the advancement of pregnancy from the first to the third trimester, the maximum incidence being in last trimester.

144. Inquiry on studies in RH sensitization under Dr. V.S. Mangalik at the K.G. Medical College, Lucknow.

Determination of Rh factor was carried out both in men and women. The tested population was divisible into four groups :—

- (a) Females who had not been pregnant.
- (b) Expectant mothers.
- (c) Females who had been pregnant.
- (d) Males.

The first group determined the incidence of Rh negative cases in the female population that were likely to be affected by disease or by Rh-positive transfusion. The second group sorted out cases who were sensitised or who were likely to be sensitized during the subsequent pregnancy. The third group determined the number of females who had already been sensitized. The fourth group was formed to meet the demand of Rh negative blood for transfusion to Rh-negative cases.

Expectant mothers attending the antenatal clinic of Queen Mary's Hospital, K.G. Medical College, Lucknow, and Government Dufferine Hospital, Lucknow, formed the subjects of this study. A record was entered of any abortion, premature delivery, or still birth in the past or present pregnancy. In those cases who delivered in the hospital, the health of the new born child at birth and in the immediate post-natal period was followed. The records of the women investigated were taken on the same lines as recommended in the I.C.M.R. proforma.

In the later stages, because of difficulty in obtaining Rh-sera, it was decided to limit the investigation to those women only who delivered in the hospital. Thus, it was possible to test with the mother her child also.

In all 2,704 persons were tested. Of these 1,985 were expectant women. Among these females, in 200 it was possible to investigate both the mother and the new-born baby.

ABO and Rh (D) blood groups were determined in all investigated cases. D-negative samples were further studied for the detection of Du antigen by the use of anti-human globulin serum. All sera obtained from Rh negative mothers, whether primipara or multipara, and whether they gave a history of repeated abortions or not, were examined for the presence of antibodies against D both in saline and protien menstrum. Attempts were made to contact the husband and children of these Rh-negative mothers for the determination of Rh (D) and ABO blood groups. However, it has not been possible to do so. Either the family members lived outside the town, or if they could be contacted in the town, they refused to give blood.

Techniques employed.—Almost all the immune sera employed by us were manufactured by Ortho-Pharmaceutical Corporation, New Jersey, U.S.A. They were of uniform high titre and acidity. At later stages sera supplied by Tata Memorial Institute of Cancer Research were employed.

Samples of blood were obtained from the vein, and all tests were carried out on clotted blood. In the new born the cord blood was tested.

(a) DETECTION OF RH (D)

Slide technique for the detection of D antigen was employed. A drop of anti-D Slide Test serum was put on a glass-slide previously warmed (surface temperature 40°C. to 45°C.). Two stick-full suspension of the cells from the clot of the blood to be tested were mixed with the serum (forming roughly a 2 per cent suspension) with the applicator. Agglutination was looked for after two minutes. All negative results were checked microscopically, and by tube method.

(b) DETECTION OF DU

A red cell suspension (2 per cent) was prepared in normal saline from the clotted blood. To two drops of this suspension in a small test-tube was added one drop of anti-D Slide Test serum. The mixture was incubated at 37°C. for one hour. After this period the cells were thoroughly washed three times, with normal saline and decanted completely after the last washing. Two drops of antihuman globulin serum were then added, the mixture shaken well and centrifuged at 800 r.p.m. for one minute. Agglutination was looked for by gentle agitation, and also microscopically.

(c) *Detection of anti-bodies in Rh-negative women.*—The following procedures were adopted in routine for the detection of antibodies in each case.

(i) Two drops of the serum to be tested was mixed with two drops of a 2 per cent suspension of Rh positive, 0 group cells in normal saline in a test-tube. The mixture was incubated at 37°C. for one hour and examined for agglutination.

(ii) The above procedure was repeated using a 2 per cent red cells suspension in 20 per cent bovine albumin solution.

(iii) *Direct Coomb's test.*—Two drops of a 2 per cent normal saline suspension of cells were washed three times with normal saline. After the last washing the cells were completely decanted off and two drops of anti-human globulin serum were added to them. The mixture was mixed well, centrifuged at 800 r.p.m. for one minute and read for agglutination both by naked eye and microscope.

(iv) *Indirect Coomb's test.*—A mixture of two drops of a 2 per cent saline suspension of Rh positive, group cells and two drops of the serum to be tested was incubated at 37°C. for one hour. Next the cells were washed three times with normal saline, and two drops of anti-human globulin serum were added. The mixture was well-shaken centrifuged at 800 r.p.m. for one minute and read for agglutination.

1. *Analysis in Rh-negative women.*—Of all the women investigated 14 were found to be Rh-negative. Half of these belonged to group 0. Of these women, 10 were admitted to the hospital. Two were cases of abortion and in the other 8 babies were born alive, were in good health and did not show any evidence of hæmolytic disease. In 6 of these mothers and their children Coomb's test both direct and indirect were negative. No circulating Rh-antibodies could be detected in the blood of these 10 women.

3. *Abortions.*—In the Rh negative group the percentage of women who gave history of abortions was significantly higher than those in the Rh positive group. In the Rh-positive group, of the 1,971 expectant mothers, in 39.2 per cent a history of abortion was elicited, while in the Rh negative group 85.7 per cent of the women gave history of abortion.

4. *New-born babies.*—In none of the new-born babies there was any evidence of hæmolytic disease due to sensitization.

145. Inquiry on the incidence of the Rh factor in pregnant mothers and its influence in the production of toxæmia, still-births and neonatal deaths under the Principal Christian Medical College, Ludhiana.

Incidence of ABO and Rh-groups.—(Total 849 ABO groupings, 851 Rh grouping. Two patients were not grouped ABO).

O	B	A	AB
264	367	155	63
(31.0%)	(43.3%)	(18.3%)	(7.4%)

Rh factor.—Out of 851 subjects, 65 were found to be

Rh negative (dd) 7.64 per cent

This is close to last year's finding (7.60 per cent)

122 women and their infants were investigated according to the ICMR schedule and in some cases the blood of the fathers was also obtained and grouped.

The women are classified as follows :

1. Rh positive (DD or Dd)

Normal primiparæ	...	...	28
Abnormal primiparæ	...	...	2
Normal multiparæ	...	...	48
Abnormal multiparæ	...	...	35

2. Rh negative (dd) women 8

'Normal' for purposes of this investigation meant a woman with no history of still-births, abortions, neo-natal deaths or toxæmia. A history of general medical disease, malpresentation or death of children after the neonatal period did not preclude a woman from being considered 'normal' in this restricted sense.

Of the 8 Rh negative women, four had normal infants. Two had children who were still-born with congenital deformities but in whom no antibody was demonstrated either in the maternal or cord blood. Two women had antibody in their sera, one a weak complete antibody, (titre 16), the second a very strong one (complete, titre in saline 1024). These two cases are described in detail in the full report.

It is considered that Rh incompatibility plays only a small part in the causation of foetal and maternal disease in the Punjab. There was no evidence of sensitisation of Rh positive mothers by Rh negative infants. ABO incompatibility between husband and wife, or mother and child, was not demonstrated as the cause of hæmolytic disease in any case.

146. Study of Rh-factor in pregnant mothers under Drs. Subodh Mitra and Santosh Mitra in collaboration with Dr. C. R. Das Gupta at the Chittaranjan Sevasadan College, Calcutta.

The object of the scheme.—The purpose of the study is to ascertain the importance of Rh-factor, if any, in neonatal deaths, still-births, toxæmias and hæmorrhagic diseases of the new-born.

MATERIALS AND METHODS

The source of material was from the Antenatal and Indoor Obstetrical Department of Chittaranjan Seva Sadan College of Obstetrics, Gynæcology and Child Health.

Total number of Rh-test done : (1.10.1956 to 30.9.1957)

	Total number of Rh-test done	Rh+ve in number	Rh+ve, per cent	Rh-ve in number	Rh-ve, per cent
Mothers	487	471	96.72	16	3.28
Husbands	39	36	92.31	3	7.69
Cord blood	409	397	97.07	12	2.93
Total ...	935	904	96.65	31	3.35

Total number of Coomb's tests done :

Negative	...	521
Positive	...	Nil

Blood groups :

Group A	...	233 (25.107 per cent)
Group B	...	314 (33.836 per cent)
Group O	...	302 (32.545 per cent)
Group AB	...	79 (8.512 per cent)

Total number genotyping done : ... 31

Incidence of abortion in 487 mothers :

Rh-positive	...	13 (2.66 per cent)
Rh-negative	...	Nil (0.00 per cent)

Incidence of still-birth in 487 mothers :

Rh-positive	...	21 (4.30 per cent)
Rh-negative	...	Nil (0.00 per cent)

Incidence of neonatal death in 487 mothers :

Rh-positive	...	9 (1.85 per cent)
Rh-negative	...	1 (0.20 per cent)

Incidence of toxæmia of pregnancy in 487 mothers :

Rh-positive	...	18 (3·70 per cent)
Rh-negative	...	1 (0·20 per cent)

Incidence of blood groups in Rh. negative mothers :

Group A	...	6 (37·50 per cent)
Group B	...	5 (31·25 per cent)
Group O	...	5 (31·25 per cent)
Group AB	...	Nil (0·00 per cent)

OBSERVATION

As the work remained suspended for about three months due to want of Rh-anti sera, expected number of cases could not be studied.

Percentage of Rh-negative mothers is 3·28, where as it was 2·7 during the last year. Percentage of Rh-negative in total individual (mothers, fathers and cord blood) is 3·3 against 3 last year.

Though the number of cases are not adequate enough, it has been found that abortions and still-births did not occur in Rh-negative mothers in this series and there was only one case each of neonatal death and toxæmia of pregnancy but the number being negligible, no valid conclusion can be drawn till more cases are studied.

147. Inquiry into the incidence and causes of still-births and neonatal deaths under Dr. (Mrs.) P.M. Naidu and Mr. V. Gopal Rao at the Osmania Medical College, Hyderabad Deccan.

Investigations carried out from 1-10-1956 to 31-9-1957.—During the year under report 194 cases of foetal loss were investigated (still-births 97, neonatal deaths 97). Out of this number 100 still-born foetuses and dead infants were available for autopsy. The clinical data were recorded on a standard case sheet. The autopsy examinations were carried out in detail and observations recorded on a separate proforma. The tissues were processed for histological examination in the usual way, special stains were applied wherever necessary and histological findings recorded.

As reported previously, in the present series again it is seen that the principal obstetric causes were trauma and stress asphyxia maternal toxæmia, antepartum hæmorrhage, foetal deformities and maternal diseases, which accounted respectively for 25·7 per cent, 23·1 per cent 10·3 per cent, 4·6 per cent and 11·3 per cent of cases. In 22·1 per cent of cases no maternal or obstetric cause could be detected. Morbid anatomical and histological examination revealed that in 27·0 per cent of cases the lesions were tissue changes associated with anoxia. In 29·0 per cent of cases pulmonary lesions were the main findings. Noteworthy among the latter were four cases of resorption atelectasis associated with

hyaline membrane. Birth injuries and congenital anomalies each accounted for 8.0 per cent. It is interesting to record that in the present series only one case showed evidence of syphilis as against 8.1 per cent in the previous series. There was no fatal case of erythroblastosis in the present series but one case of neonatal jaundice was observed and proved to be due to Rh-incompatibility. This baby recovered on appropriate treatment. Twenty-five per cent of the foetuses were macerated. But of these again in only 2 the cause of death remained obscure. It will thus be seen that anoxia and pulmonary lesions accounted for a large majority of cases of foetal loss.

Investigations for Rh-factors.—During the year under report a total of 171 cases were investigated for Rh factor. These were :—

1. Antenatal cases Rh tested

Rh positive (anti-D)	...	133
Rh negative (anti-D)	...	15
		148

2. Cases of still-births and neonatal deaths investigated (mother's blood tested).

Rh positive	...	19
Rh negative	...	2
		21

Of the two Rh-negative mothers the infants of neither showed any evidence of erythroblastosis foetalis, death being due to unrelated causes in both.

3. *Neonatal jaundice.*—Two cases of this condition were investigated for Rh-factors. Only one of these was proved to be a case of Rh incompatibility. The jaundice was mild and the infant recovered on suitable treatment.

Survey of socio-economic conditions of the affected mothers.—In the course of the present study the Health Visitor attached to the inquiry visited 252 mothers in their homes and recorded data on housing, sanitation, the family size and income. She has also carried out the diet surveys on these families. All these data are now being analysed. The general impression is that in a large majority of these cases the socio-economic conditions are unsatisfactory, and their nutrition status is low. The food intake per consumption unit worked out to less than 1,200 calories and the diets were grossly deficient in protein and vitamins.

148. Inquiry into the causes of perinatal deaths under Drs. A. Bhowmick and S. De at the Medical College, Calcutta.

The object of the work is determination of the exact causes of perinatal deaths by post-mortem examination including histological

examination of selected tissues. The term perinatal death in this investigation has been used to mean antepartum and intrapartum foetal deaths and neonatal deaths in the first 10 days of life. It is expected that this work will ultimately help in discovering the preventable causes of death and the steps that should be taken towards reduction of foetal and neonatal deaths.

During the year under review (from 1.10.1956 to 30.9.1957) it has been possible to make another 184 post-mortem examination of which 77 were on still-born foetuses and 107 were on live born babies that died in the first 10 days of life. The autopsy and histological findings revealed the following causes of still-births and neonatal deaths.

TABLE A
Causes of still births (fresh).

	Premature	Per cent	Mature	Per cent
(i) Trauma intra-cranial Hge. intra-cranial stress.	3 0	10·3	13 4	27·0 8·3
(ii) Intra uterine asphyxia	19	65·5	26	54·2
(iii) Developmental defects.	3	10·3	1	2·0
(iv) Pneumonia	1	3·4	1	2·0
(v) Undetermined.	3	10·3	3	6·2
Total :	29		48	

TABLE B
Causes of neonatal deaths.

	Premature	Per cent	Mature	Per cent
(i) Trauma intra-cranial Hge. intra-cranial stress.	7 5	9·7 6·7	2 1	6·0 3·0
(ii) Asphyxia	12	16·0	10	33·3
(iii) Pneumonia	27	36·5	11	33·3
(iv) Hyaline membrane	11	15·2	1	3·0
(v) Atelectasis	5	6·7	2	6·0
(vi) Developmental defects	1	1·3	1	3·0
(vii) Hæmolytic diseases	2	2·7	1	3·0
(viii) Undetermined	4	5·4	3	9·0
(ix) Pyopericardium	0	0	1	0
Total :	74		33	

It may be stated that while the general causes of still-births and neonatal deaths remain the same as those observed by workers in other countries, the incidence of the different causes varies. In this series the incidence of pneumonia appears to be higher and that of hyaline membranes lower than those observed by British and American workers.

149. Inquiry into the relationship between deficiency of vitamin 'B' complex and toxæmias of pregnancy under Drs. M.L. Pai and T.V. Patel at the Medical College, Baroda.

Toxæmias of pregnancy forms a major problem to obstetricians, not only in India but in other countries as well. Maternal as well as foetal mortality, resulting from it, is a very common phenomenon. Thus, as a cause of maternal and foetal mortality, it ranks next to anæmias in this country. Nutrition plays an important part in the causation of this condition. It is of greatest importance to know whether the dietary habits of the pregnant women are adequate for their needs, particularly because of the fact that the growing foetus makes extra demands upon their requirements. Nutritional disturbances in the mother associated with low vitamin intakes may be one of the etiological factors in vomiting, eclampsia, etc.

Excretion of thiamine, riboflavin and nicotinic acid in 24 hour collection of urine, both before and after the test-dose of each of these vitamins, was studied in cases of toxæmias of pregnancy. These cases have been studied both before and after treatment. The collection of urine was analysed for these vitamins in these cases once before delivery and after delivery on 1st-2nd, 4th-5th and 9th-10th days. For the comparative data required, one series of normal female healthy subjects was studied for their urinary excretion levels of those vitamins. Another series of normal pregnancy cases was also studied similarly.

In the preliminary work done, urinary excretion of nicotinic acid in twenty normal healthy subjects was determined. In continuation of this preliminary work, fifteen cases of toxæmias of pregnancy were studied for determining the excretion levels of nicotinic acid in their 24-hour collection of urine. The excretion of nicotinic acid in these cases was found to be less (about 50 per cent) when compared with the corresponding vitamin excretion in normal subjects.

A series of normal healthy female subjects was studied for the daily urinary excretion of the above vitamins both before and after the test-dose of the respective vitamin was given. Another series of normal pregnancy subjects was studied, as the similar comparative data were required for comparison with the levels of excretion of these vitamins in 24 hour collection of urine obtained from the toxæmia cases. Toxæmia cases were studied on similar lines. Their daily urinary excretion of these vitamins was determined both before and after the test-dose on their admission to the wards. These cases were studied both before and after treatment. Significant results have been obtained showing a definite tendency towards the deficiency state of saturation of the tissues in the body of

these cases with respect to these vitamins, particularly nicotinic acid & thiamine, when compared with the normals. There is a tendency towards getting this deficiency state lessened with the stage of recovery proceeding in the step-by-step stages as seen from the results obtained of the estimations done on the different days 1st-2nd, 4th-5th and 9th-10th after delivery.

150. Study on chlorpromazine in the field of obstetrics in toxæmia and as obstetric analgesia under Dr. S.C. Bose at the Medical College, Eden Hospital, Calcutta.

The object of this inquiry is to study the effect and values of chlorpromazine as an analgesic in labour and for control of toxæmia of pregnancy by clinical trial on selected cases. Such knowledge is likely to be useful towards effecting a painless child-birth and in preventing toxæmia with its associated complications.

Forty cases in late first stage of labour were studied to see the analgesic effect of chlorpromazine and the course of labour thereby modified, if any, as regards the duration of labour, method of delivery, nature of uterine contraction and the effect on the maternal and fetal conditions.

Twenty case (14 eclampsia and 6 pre-eclampsia) of toxæmia of pregnancy were studied to assess its value regarding toxæmia and diminution of maternal complication and risk to fetus.

RESULT IN BRIEF

In obstetrics analgesia :

1. Chlorpromazine hydrochloride in combination with other drugs, such as pethidine hydrochlor or promethazine hydrochlor or even alone in some cases produced good amnesia-analgesia effect in 14, fair in 17 and poor in 6 cases, respectively. Three cases where uterine inertia, developed have been excluded.

2. No untoward effect was reflected on the mother or fetus. There was only one fetal death in the series, the cause being other than chlorpromazine therapy.

3. The courses of labour were little affected, particularly the 3rd stage which ended within 10 minutes in each case and there was no rise in the incidence of P.P.H.

In some cases however, uterine contraction became sluggish and 3 of these cases developed uterine inertia Pitocin I.V. drip improved this condition of inertia and at present use of pitocin drip with chlorpromazine hydrochlor is under study.

In toxæmia :

1. Chlorpromazine alone has got very little effect in controlling the fits but potentiated action of other drugs, such as pethidine hydrochlor and/or promethazine hydrochlor with chlorpromazine has got promising effect.

2. It is possible in some cases, may be due to individual susceptibility, that the B.P. came down suddenly even with a small dose of chlorpromazine.

3. Hyperpyrexia in eclamptic patients could not be controlled with chlorpromazine.

151. Clinical and hormonal studies in women with primary and Secondary Amenorrhoea under Dr. L.V. Phatak at the G.R. Medical College, Gwalior.

Thirty-one cases with a complaint of amenorrhoea were investigated in 3½ months period from June to September 1957.

Studies and investigations.—As a routine, complete blood picture with E.S.R., W.R. or Kahn test, urine examination, endometrial biopsy and hysterosalpingography were done in all cases. In some cases, additional investigations, e.g. x-ray of the chest and B.M.R. were also done, when felt necessary.

DISCUSSION

The cases were considered under 3 groups—those of primary amenorrhoea, cases of secondary amenorrhoea with no pregnancy and the parous group with secondary amenorrhoea.

The age incidence in cases of primary amenorrhoea had an average of 20-25 years. In the group of secondary, amenorrhoea, it was 21 years in nulliparas and had an average of 25-37 years in parous group.

Average age of menarche is higher in nulliparas group, i.e. 14-17 years, whereas it is 13-25 years in parous group. In the nulliparas, out of 11 cases 6 had a normal menarche, while 5 had a late menarche with an average of 16 years and 2 months. Out of 16 cases of parous women, 13 had a normal menarche, while 3 had a late menarche with an average of 15 years and 4 months. In these 13, with normal menarche history of hypomenorrhoea was present from the onset of menarche sometime or the other.

Adenexal masses were present in 10 cases. Of these, 1 showed tubercular endometritis, another sarcoidosis and 1 revealed presence of foreign body giant cells.

Remarks.—Infectious fevers appeared to be influential in menstrual history. An association of tubo-ovarian masses needs careful investigations to eliminate a tubercular lesion.

A late menarche associated with nulliparity appears to precipitate amenorrhoea at an early age. A history of hypomenorrhoea appears to be noticeable in the parous group that have developed secondary amenorrhoea later on.

Thus, some definite indications in the history appear to be present before secondary amenorrhoea actually precipitates. What exactly precipitates a late menarche or a subnormal function of menstrual system needs further clarification.

152. Inquiry on hormone assays for pregnanediol œstrogens, chorionic gonadotrophins and 17-ketosteroids under Dr. Subodh Mitra at the Chittaranjan Cancer Hospital, Calcutta.

Thirty-eight samples of urine have been assayed for œstrogens in normal pregnancy and 9 samples in toxæmic pregnancy. During the same period 34 samples of urine have been assayed for pregnanediol in normal pregnancy and 8 samples in toxæmia. In all we have studied three individual cases week by week throughout pregnancy and after confinement.

The technique followed for pregnanediol estimation is that of Sommerville, Gough and Marrian (1948). For estimation of œstrogens Stevenson and Marrian's method (1947), modified by Brown and Braunsberg (1954) has been followed. The three fractions of œstrogens (œstrone, œstradiol and œstriol) have been separated by Column Chromatography using Celite-535, the temperature being maintained between 25°C. to 27° C. and then estimated calorimetrically by Kober reagent.

Our average result for pregnanediol has been found to be 43·20 mg./24 hours (32 weeks) in normal pregnancy and 37·76 mg./24 hours (37 weeks) in toxæmic pregnancy against Cope's (1940) result : 55 mg./24 hours and Venning's (1937) result : 75 mg./24 hours in normal pregnancy. The average result for œstrogens has been found to be 18·76 mg./24 hours (33 weeks) in normal pregnancy and 9·09 mg./24 hours (33 weeks) in toxæmic cases, against Venning's (1937) result : 21 mg./24 hours (40 weeks) in normal pregnancy.

It has been observed that there is more or less a steady rise of pregnanediol and œstrogens levels with the progress of pregnancy followed by a fall prior to labour in normal cases. The pattern of rise and fall is similar in toxæmic cases though the excretion of both is lower than in normal cases.

M E N T A L H E A L T H

- 153.** Inquiry designed to provide basic biochemical and hæmatological data associated with mental disorders under Dr. M.V. Govindaswamy at the All-India Institute of Mental Health, Bangalore.

Investigations on the hæmatological and biochemical constituents have been carried out on patients admitted to this hospital with siblings or near relatives as controls. Data have been obtained on 60 random selected patients with their corresponding controls during the period April to September 1956, and about 400 in-patients of the hospital during the period November 1956 to September 1957.

The following are the aspects of investigation :—

I. Complete hæmatology including :

- (a) Red and white blood cell counts.
- (b) Specific gravity.
- (c) Hæmoglobin.
- (d) Packed cell volume.
- (e) E.S.R.

II. Biochemical constituents :

- (a) Blood sugar.
- (b) Total Proteins and A/G ratio.
- (c) Total non-Protein nitrogen.
- (d) Sodium, Potassium and Calcium.
- (e) Chlorides and Inorganic Phosphorus
- (f) Cholesterol.
- (g) Acid and Alkaline Phosphatases.

III. Electrophoretic separation of serum proteins using the agar-gel electrophoretic method, the patterns of serum protein fractions of selected, chronic and freshly admitted schizophrenic cases have been investigated.

Apart from the above investigations associated with the inquiry, work in the following directions has been carried out.

(A) Complete examination of C.S.F. including cells, sugar chlorides, proteins and Lange's colloidal test have been carried out in 200 samples of C.S.F. of patients belonging to different types of mental disorder.

(B) The amino-acid patterns in the urinary and faecal excretions and also in the cerebrospinal fluid of mental patients have been investigated in selected cases, with the help of paper-chromatographic method of amino acid analysis. Changes of the amino-acid patterns in the body fluids of the mental patients as against controls are being studied in detail to understand variations.

154. Inquiry into the psychological factors related to adolescent adjustment under Dr. T. K. N. Menon, Dean, Faculty of Education and Psychology, University of Baroda, Baroda.

Emerging into adolescence, the child suddenly becomes unbalanced, unpredictable and unstable. While this presents tantalizing problems both for parent and educator, the clinician finds in the adolescent certain personality, behaviour, and character disorders quite different from those of the adult and the child. To understand the complexity of adolescent personality and its functioning, the present inquiry was taken up.

Adolescence is understood as a period of transition from dependence upon adult direction and protection to self-dependence and self-determination. It is a period of constant 'stress and strain' though many young persons outgrow this phase as healthy. Patterns of dynamic adjustment are limited for any given person, first by the increasing disposition rigidity or the functional autonomy of drives with age, second by the fixed temperamental and ability dimensions of personality with which dynamic traits have to operate, and thirdly, by fixity of the cultural environment to which the person has to adjust. Keeping in view the vagaries of Indian life, the changing political and economic conditions, the effects of industrialization and urbanization, the structure of society, beliefs and convictions, the impact of Western influence, emphasis is laid to study the adolescent adjustment on the Indian spectrum.

To study various psychological variables, adjustment has been conceived as a function of certain psychological factors, such as intelligence, personality variables, attitudes and interests, environmental stimuli and drive patterns. The research programme is split into four stages. They are :

- (1) Construction of adjustment questionnaires ;
- (2) Study of personality traits of the adjusted and mal-adjusted adolescents ;
- (3) Study of differences in the environmental characteristics of the two groups ;
- (4) Attitudes and interests analysis of the criterion groups.

The present stage of study corresponds to the construction of the adjustment questionnaires in four areas. They are :

- (1) Social adjustment.
- (2) Family adjustment.
- (3) Emotional adjustment.
- (4) Occupational orientation.

The inventory with regard to social adjustment is ready, and norms nearly developed. Besides the factor of social learning, the emphasis is placed upon the variety and variability of the processes of physiological maturing and upon the complexity of the influences of boys and girls upon one another, and the subtlety of their inter-relationships with the adult groups of which they form the fringe. The inventory has been administered to a sample population of about 1,100 adolescents in the age group of 12 to 18.

A very comprehensive approach is made to study the family adjustment. The family is considered as a socio-emotional learning ground and as a shelter. Attention is paid to the intra-familial attitudes, economic status, size of family, personality characteristics of parents, the interaction of the family with the neighbourhood, sibling relationship, etc. The inventory has been administered to a group of 370 high school adolescents. To determine the validity of items in terms of an external criterion, work is going on for selecting a certified sample of family adjusted and mal-adjusted individuals.

The inventory with regard to emotional adjustment is ready, and the pilot study is being done. Emotional adjustment is considered not in terms of their control, but their modification in the nature of their stimuli and in the type and immediacy of the response made to such stimulating circumstances. About fourteen hypothetical variables, both in terms of symptomatic and intrinsic manifestations are considered in the formation of the inventory.

Exploratory work with regard to the occupational orientation and personality traits is also at hand. The study, when completed, would be of immense value specially from the point of view of clinical assessment and thus providing therapeutic aid, guidance and counselling to our youth.

155. Study of the carbohydrate metabolism in mental patients under Dr. D.N. Nandi at the Nilratan Sircar Medical College, Calcutta.

Fifty patients, including 42 males and 8 females, suffering from different mental disorders, e.g., schizophrenia (31), mania (6), depression (5), paranoia (8), and 10 normal subjects were studied.

Oral and intravenous glucose tolerance tests and insulin sensitivity tests were performed in all these 60 subjects. Oral glucose tolerance test

was repeated in 8 patients suffering from schizophrenia, when they either recovered or considerably improved after E.C.T. or insulin therapy.

It appeared from the result so far obtained that fasting blood sugar level in patients suffering from schizophrenia was slightly lower and in patients suffering from mania and depression it was slightly higher than normal controls. Patients suffering from schizophrenia, mania and depression showed prolonged oral glucose-tolerance curves; but the curves tended to become normal when the mental condition of the patients improved. Intravenous glucose-tolerance curves were normal in all groups of subjects. So, the prolonged oral glucose tolerance curves might be due to delayed absorption from the alimentary canal. Some hormonal factors might be responsible for such delay in the absorption of glucose. Insulin sensitivity test did not show any abnormality in any group of cases under investigation. Patients suffering from paranoia, did not show any significant variation in these tests from that of normal controls. But as the number of cases studied was small no definite conclusion could be made.

156. Inquiry on electro-encephalographic and electro-corticographic studies with the help of specially designed magnetic pick up analyser under Dr. M.V. Govindaswamy and Shri R.L. Narasimhaiya at the All India Institute of Mental Health, Bangalore.

Electro-encephalographic recordings are being taken as a routine with a view to studying the effects of hyperventilation and photic stimulation in the records, in addition to the taking of resting record of a number of patients referred to the department, as well as of hallucinated schizophrenics selected from the Mental Hospital.

With a view to analysing the effects of various physical stimuli on the record, a magnetic recording drum has been designed. The drum is now under construction in a local workshop in accordance with the specifications supplied to them.

A power pack and an amplifier for the magnetic recorder have been designed. Most of the components for these circuits have been secured and the apparatus is under construction and is expected to be ready in a short time.

Magnetic recording coils have been designed and constructed.

157. Inquiry on psychomotor epilepsy under Dr. E.P. Bharucha at the Seth G.S. Medical College, Bombay.

Electro-encephalogram.—A total of 34 E.E.G. have been recorded on the 25 cases. Apart from the routine record and photic activation, the special sphenoidal electrodes have been used (under pentothal anaesthesia) as described by Pampiglione.

Radiological investigations.—Pneumo-encephalograms have been done on 8 patients. Bilateral cerebral angiograms have been done on 7 patients.

Treatment.—Some of the patients who were already on various types of anti-convulsant medication before taking up for study and who showed good response to the medication are continuing to take the same medication. The new cases are put on either Mesantoin or Primidone (Mysoline).

NUTRITION

158. Nutrition Research Laboratories, Coonoor.

A detailed account of the researches in progress will be found in the annual report of the Nutrition Research Laboratories for the year 1956-57 which is being published separately. The following account deals briefly with the salient features of the investigations and other activities.

I. STUDIES ON VITAMINS

Dietary protein and vitamin A in liver.—The effect of different levels of dietary protein on vitamin A storage in liver was studied in young rats receiving small doses of vitamin A. Maximum liver storage was observed at 12 per cent protein as casein. With β -carotene, high levels of casein upto 30 per cent promoted better storage of vitamin A.

Effect of vitamin D on liver blood, and epiphyseal cartilage of rachitic rats.—The administration of a single large dose of vitamin D to rachitic rats brought about a marked increase in (a) liver citrogenase; (b) plasma citrate and (c) calcium and phosphorus in the epiphyseal cartilage.

Starvation and rickets.—Healing induced by acute starvation was not accompanied by increases in citrogenase activity or citrate in the epiphyseal cartilage although the total P in cartilage increased progressively. Histochemically, a non-calcific phase of mineralisation of the cartilage was demonstrated to occur in healing induced by starvation. This phase was followed by calcification in rachitic animals treated with vitamin D.

II. STUDIES ON PROTEINS

Vegetable protein and regeneration of blood proteins.—Experiments on protein depleted rats fed a diet in which proteins were derived from a mixture of cereal, pulse and leafy vegetable have shown that vegetable proteins are capable of effecting full regeneration of haemoglobin and plasma proteins within three weeks. The rate of regeneration was however, slower in the earlier periods as compared to that observed on skim milk protein.

It was further observed that blood transaminase activity which was depressed in protein deficiency was also restored to normal within 14 days of repletion.

Blood transaminase (G O T) in protein depletion with and without calorie restriction.—In rats on the adequate protein—no pyridoxine diet blood G O T activity decreased significantly after 28 days. On the other hand, in animals on the low protein + pyridoxine diet, the enzyme activity decreased to a greater extent. The apoenzyme found to respond rapidly to changes in the levels of dietary protein whereas the effect on the co-enzyme due to changes in the intake of pyridoxine was more gradual.

The superimposition of calorie restriction on protein and pyridoxine deficiencies had no beneficial effect on blood G O T activity.

Co-enzyme I in protein deficiency.—In protein deficiency alone the total DPN showed a fall within a fortnight; the fall in DPN was relatively greater than in DPNH resulting in a low DPN : DPNH ratio. In the combined deficiency of protein and choline both the components were reduced in almost equal proportions with the result that the DPN : DPNH ratio remained similar to that in the positive controls.

DPN-ase in liver.—DPN-ase activity in liver was reduced in protein deficiency, the activity being further depressed by the superimposed choline deficiency. It was concluded therefore that the low levels of DPN in livers of protein deficient animals were not due to an increased breakdown of DPN in liver.

Effect of protein and choline deficiency on some oxidative enzymes in liver.—Malic oxidase activity of the liver homogenate of rat was greatly decreased in protein and/or choline deficiency when DPN was not added to the reaction mixture. The addition of DPN enhanced malate oxidation although the levels were still lower than the values given by livers from the control animals.

In protein deficiency as well as in the combined protein and choline deficiency the activity of DPNH-cytochrome c reductase in rat liver was reduced by about 50 per cent within a week.

III. STUDIES ON IRON METABOLISM

Iron balance in human subjects.—Observations on four healthy young male volunteers fed fully on vegetarian diets or those containing meat and fish showed that on an intake of 38 to 44 mg. Fe daily, 7 to 8 mg. Fe was retained. No significant difference in Fe balance between the two diets could be demonstrated.

IV. STUDIES ON ENERGY METABOLISM

The energy cost of some activities under non-basal conditions was determined in 11 subjects. The average values in Cal./kg./hr. were: Sitting on chair, 1.31; Sitting on chair writing or working at calculations,

1.60; Standing at ease, 1.40; Standing doing laboratory work, 2.05; walking at 3 m.p.h. on level smooth surface, 3.43.

V. NUTRITION CLINIC

During the period 1st August, 1956 to 31st July, 1957, 4,704 cases attended the clinic. Of these 240 cases were found to be suffering from nutritional disorders; 83 cases were of kwashiorkor, 61 of vitamin A deficiency and 54 of vitamin B-complex deficiency.

VI. CLINICAL INVESTIGATIONS

Protein malnutrition

The validity of the urea method for the estimation of total body water in nutritional oedema.—Total body water was determined by the urea and antipyrin methods in apparently healthy subjects and in nutritional oedema. The results were comparable showing that the urea method is reliable.

Follow-up study of 15 cases of kwashiorkor.—Fifteen children between the ages of 2 and 10 years who had suffered from kwashiorkor at periods ranging from nine months to eight years before the present investigation were re-examined and liver biopsy performed. The results showed that fatty infiltration of the liver as well as fibrosis was absent in all cases. Kwashiorkor is, therefore, not necessarily followed by permanent or progressive hepatic fibrosis eventuating in cirrhosis.

Ferritin in nutritional oedema.—The presence of ferritin was demonstrated in the blood of five of the six children with kwashiorkor and two adults with nutritional oedema at the time of admission. In all cases ferritin was absent after a month of high protein therapy.

The output of breast milk in different stages of lactation.—Observations on 24 hours output of breast milk were made upto 90 weeks by test feed technique on 14 mothers aged 20 to 30 years belonging to the poor socio-economic group. The results revealed that although the yield of milk registers a decline after the first year, considerable amounts continue to be secreted well beyond this period.

Effects of dietary protein supplementation on lactation.—Observations on 6 mothers fed-controlled diets indicated that an increase in protein intake from 60 g. to 100 g. daily resulted in an appreciable increase in the 24 hour output of breast milk, accompanied, however, by a diminution of protein concentration in milk.

Nitrogen balance studies in lactating women.—Nitrogen balance determinations on intakes of 60, 100 and 115 g. of protein were carried out on the above subjects during the period of their hospitalisation. A linear relationship was observed between nitrogen intake and nitrogen retention ($y=0.5363x-0.1294$) on the one hand, and between N absorbed and N retained ($y=0.5806x-0.0876$) on the other.

NUTRITION IN TUBERCULOSIS

Experimental.—Experiments with guinea pigs and rats showed that the effect of different levels of protein intake on induced tuberculous infection as judged by the percentage of animals infected in each group, the extent and intensity of the lesions, and by the survival rate was not significantly different. However, among rats on the vitamin A deficient diet, the percentage of animals infected and the extent and severity of the lesions were appreciably greater than in the control group.

Clinical.—Results of the metabolic study with eight human cases of tuberculosis indicated that there was considerable retention of nitrogen, calcium and phosphorus on diets containing 80 g. protein or more.

Epidemiological.—Mantoux tests were done on 540 children of ages from 1 to 10 years suffering from various types of advanced malnutrition and on apparently healthy children of comparable ages. No significant difference with regard to the incidence of Mantoux-positivity between the malnourished and the control groups was observed.

DIET AND SERUM CHOLESTEROL

Epidemiological.—Serum cholesterol levels were determined in 447 healthy men belonging to different socio-economic groups. The findings were : (i) the values for serum cholesterol among the poor were considerably lower than those reported for European and American population; (ii) in all groups, serum cholesterol values tended to increase from the 20th to about the 35th year; (iii) after about the 35th years, the poor groups had a stationary or declining serum cholesterol but in the well-to-do, the level tended to rise progressively with age.

Experimental.—The effects of ingestion of high levels of different types of fats were investigated in human volunteers for a period of seven weeks and in monkeys for 6 to 12 months. The results showed that those receiving hydrogenated vegetable fat showed a marked rise in serum cholesterol whereas in those receiving sesame oil, the values were either unchanged or were lower than the initial. Almost similar results were obtained in monkeys.

Studies on the blood of poor Indian new born babies and their mothers. Cytometric determinations together with the estimations of serum proteins and their fractions, calcium, total and ester cholesterol were made in the venous blood obtained from mothers and their new born babies. In spite of the relatively poor nutritional status of the mothers, the haematological values for the new born obtained in the present study were in close agreement with the values reported elsewhere.

VII. DIETETICS

Survey of dietary habits of lactating and pregnant mothers.—A careful and detailed oral-questionnaire diet survey in 100 pregnant and 70

lactating women belonging to the poor socio-economic groups showed that diets in pregnancy and lactation were no different from those consumed by these women in other periods.

VIII. PATHOLOGY

Nutritional and hormonal factors governing mesenchymal reactions in the liver.—A spontaneous regression of dietary cirrhosis has been observed in the livers of rats maintained on choline-free, high-fat, low protein diet for five months. In chronic CCl_4 poisoning, it was observed that rats on 20 per cent protein diets developed more severe hepatic fibrosis than those on 2 per cent protein diets. A deficiency of dietary protein probably has an inhibitory effect on fibrous tissue formation in spite of chronic liver damage.

Study of the pathology of "Infantile Cirrhosis".—The material for study consisted of liver biopsy specimens obtained at different stages from a large number of cases of "Infective Hepatitis". "Infantile Cirrhosis" and "Hepatic enlargement of asymptomatic siblings", obtained through the kind courtesy of Prof. S.T. Achar. The pattern of histological changes in the liver in these conditions closely resembled the several changes that occur at different stages of Infective Hepatitis in adults.

IX. FIELD INVESTIGATIONS

Protein malnutrition survey in South India.—During 1956-57, the multi-phasic protein malnutrition survey on infants and children under 5 years of age from the poor socio-economic groups was extended to several other areas in South India. The areas surveyed and the numbers examined were Andhra (507) ; Madras (823) ; Mysore (518) ; and Kerala (578).

The investigations comprised (i) a survey of economic and living standards of the people ; (ii) a study of their general food habits, with special reference to the infant feeding and weaning practices ; (iii) an estimation of the dietary intake, and (iv) an anthropometric, clinical and biochemical examination of children with particular reference to protein nutriture.

The surveys included clinical examination with particular reference to the signs and symptoms of kwashiorkor in 2,426 children under 5 years of age, and estimations of haemoglobin, total serum proteins, albumin and globulin in a sample of 400 children. Some of the salient features of the surveys are described below in brief.

Weaning and supplementary feeding.—Children continue to be breast fed for prolonged periods sometimes even upto the third year of life. Rice was the commonest supplementary food encountered in the various areas surveyed. Animal milk was very poor second. The total calories and proteins derived from breast milk and supplementary foods taken together were found inadequate at all ages upto 3 years.

Signs of protein malnutrition.—The incidence of various signs in 2,426 children examined was as follows : dyspigmentation of hair 313, liver enlargement of two fingers and over 81, moon face 109, oedema 24 and crazy pavement dermatosis 2.

Haemoglobin and serum proteins.—The average haemoglobin levels were invariably low (9.6 to 12.2 g. per 100 c.c.). The average values for serum protein varied within the range as follows : total protein 6.0 to 6.8 g. albumin 2.8 to 3.9 g. and globulins 2.6 to 3.3 g. per 100 c.c.

Frequent occurrence of cases of kwashiorkor among the communities, extreme growth retardation of the children examined and the biochemical alterations in the blood, etc., suggest that protein inadequacy is not only extensive, but also chronic.

X. SECOND FIVE YEAR PLAN PROJECTS

During the year under report, work on a large number of research projects sponsored by the Indian Council of Medical Research under the Second Five Year Plan was initiated. A few of these projects are located at the Nutrition Research Laboratories.

I. *Clinical trials with protein rich foods.*—The following three protein food mixtures have been taken up for investigation in the first instance : (a) Defatted ground nut flour—roasted Bengal gram powder—lucerne powder, (b) Defatted ground nut flour—roasted Bengal gram powder—sesame flour—lucerne powder, and (c) Roasted Bengal gram powder—skim milk.

II. *Studies on human lactation.*—Studies are currently in progress on (i) chemical composition of human milk in different stages of lactation, (ii) Haematological studies in lactating women, and (iii) studies on attitudes towards lactation in different socio-economic and cultural groups.

III. *Protein malnutrition survey.*—In addition to the surveys mentioned earlier, units have been established in Andhra and West Bengal to carry out further surveys and to test methods of prevention.

IV. *Field trials with protein rich foods.*—Feeding trials on 150 children of less than 5 years in age and belonging to the poor socio-economic groups have been recently launched. Groups of 50 children each were fed with following foods respectively : (i) Bengal gram—skim milk mixture ; (ii) defatted ground nut flour—Bengal gram—lucerne powder mixture, and (iii) skim milk.

V. *Studies on growth and development of children.*—So far 750 children from a rural area have been examined in accordance with the recommendations of the Working Party constituted for this purpose. The work is being extended to other rural and urban areas in South India.

XI. NUTRITION IN RELATION TO FACTORY ENVIRONMENT

A research project on nutrition in relation to factory environment in textile industry has been undertaken. During the year under report.

two Units—one in Ahmedabad and one in Coimbatore—have started functioning. Diet and nutrition surveys of the workers and their families have been undertaken as a preliminary stage in the investigation. Nutrition surveys have so far been completed on 400 workers in Ahmedabad and 700 workers in Coimbatore. Diet and socio-economic surveys have been carried out in about 100 families in each area. Further work is in progress.

XII. EDUCATION AND ADVISORY WORK

1. *Enquiries from private and governmental agencies.*—143 requests from the public and 92 requests from governmental agencies for information and advice on different aspects of diet and nutrition were received and dealt with.

2. *Annual training course in Nutrition.*—The Annual Training Course in Nutrition was held from the middle of March till the end of June 1957. Among the trainees was a candidate from Indonesia sponsored by the World Health Organisation.

3. *Seminar on protein malnutrition.*—A seminar on Protein Malnutrition was conducted from 3rd December 1956 to 19th December, 1956.

4. *Training of scientists and technicians for other institutions.*—During the year a number of scientists and technicians from different institutions spent short periods of observation, or underwent training for short periods in different departments of the Laboratories.

5. *Preparation of publicity material.*—Charts and posters which could be used as publicity material in the field of nutrition for public education have been prepared and the work is being extended.

159. Nutrition Research Unit under Dr. B.C. Guha at the University College of Science and Technology, Calcutta.

I. *Studies on the biosynthesis of ascorbic acid.*—It has been reported previously that when rat liver homogenate is incubated with pyruvate under suitable conditions, an increase in ascorbic acid content is obtained over that without the added substrate. It is, however, noticed that in no case the ascorbic-acid content after incubation exceeds that present in the tissue before incubation.

A system has been developed in which a marked synthesis of ascorbic acid takes place when glucurone is incubated with rat liver homogenate or extract (after sedimentation of the nuclear and mitochondrial fractions) in presence of potassium cyanide ($5 \times 10^{-2}m$). A variety of compounds, other than glucurone, have also been examined but without effect. Livers from other species have also been studied. Goat liver appears to be a potent source of the enzyme system concerned in this biosynthesis. An enzyme concentrate which brings about the synthesis of

ascorbic acid from glucurone in presence of cyanide has been prepared from goat liver. The aforesaid biosynthesis of ascorbic acid has been found to be inhibited by ATP, UTP and also by reagents, such as HgCl_2 , and *p* chloromercuribenzoate (P.C.M.B.), which react with the thiol group. The inhibition by PCMB can be reversed by reduced glutathione indicating that thiol-containing enzyme or enzymes are involved.

II. *Studies on the bound form of ascorbic acid.*—The presence of bound ascorbic acid was reported last year. About two tons of cabbage were worked up for the isolation of ascorbigen. The cabbage was cut into small pieces, passed through meat mincers and the juice extracted by a screw press. The ascorbigen was adsorbed from the juice by charcoal and eluted by a mixture of chloroform and alcohol. The eluate was concentrated by distillation under vacuum. Attempts are being made to isolate pure ascorbigen by partition chromatography on cellulose powder and by other processes. A material has been obtained which contains about 130 mg. ascorbigen (in terms of ascorbic acid) per g. of total solids.

III. *Biochemical studies on nicotinic acid.*—The bound form of nicotinic acid, called 'niacinogen', has been isolated from rice and wheat brans in a crystalline form. The work is now directed to the isolation of 'niacinogen' from other biological sources.

IV. STUDIES ON IRON METABOLISM :

1. *Electrophoretic studies on avian hæmoglobins.*—The hæmoglobins of 95 specimens of birds among different orders, such as *Anseriformes*, *Ciconiiformes*, *Coraciiformes*, *Columbiformes*, *Caculiformes*, *Falconiformes*, *Galliformes*, *Gruiformes*, *Passeriformes*, *Piciformes*, *Psittaciformes* and *Strigiformes*, have been studied. It has been observed that the hemoglobins of the birds under the following orders, *Cinconiiformes*, *Columbiformes*, *Coraciiformes*, *Cuculiformes* and *Psittaciformes*, contain a single hæmoglobin, whereas birds under the remaining orders contain two components. No marked differences were found among the families within an order and among the genera within a family except in two cases so far examined. 'Hill-Myna' under *Psittaciformes* and 'King fisher' under *Coraciiformes* contain two hæmoglobin components instead of one.

2. *Isolation of ferritin from normal guinea-pig liver.*—The ferritin, an iron protein complex was isolated as hexagonal crystals from the sub-cellular fractions of the normal guinea-pig liver. It was found to be located in the fraction containing microsomes and the soluble supernatant.

3. *Distribution of iron and copper in sub-cellular fractions of the normal guinea-pig liver.*—In course of this investigation it has been observed that the iron and copper contents were highest in microsomes and the soluble supernatant and least in mitochondrial fractions. The amount of copper strictly follows that of iron in increasing order in the following fractions, viz. mitochondria, nuclear cell debris, microsomes and soluble supernatant.

V. *Studies on the retention of ascorbic acid during blanching of food-stuffs.*—Studies were continued this year to prevent the loss of ascorbic acid during blanching of foodstuffs. A few more foodstuffs were studied this year and it was observed that the retention of ascorbic acid was best when the foodstuff was blanched in acid medium (lactic acid, pH 3.5). Sodium sulphite was the next best for retention of ascorbic acid. Blanching in acid medium adversely affected the colour, whereas sulphite-blanching improved the colour. Dipping in sodium sulphite with or without sodium chloride had also a beneficial effect on the retention of ascorbic acid.

VI. *Studies on the effect of cooking with different volumes of water on the in vitro digestibility of rice.*—Five pure-bred strains of rice received from different Agricultural Research Stations of different States were cooked with (a) 6 or 8 volumes and just (b) sufficient water and the cooked rice was incubated with takadiastase and pancreatin for 1 hour. The amount of reducing sugar produced after 1 hour's incubation was determined. It was observed that the rice cooked with just sufficient water yielded less sugar, whereas the rice cooked with 6 volumes or 8 volumes of water yielded more sugar.

VII. *Riboflavin and nicotinic-acid contents of some pure-bred strains of pulses.*—Riboflavin and nicotinic acid contents of some pure-bred strains of pulses were reported last year. Some of the strains were obtained from the next crop and from the same Agricultural Research Stations. They were analysed for the riboflavin and nicotinic acid contents. It was found that the riboflavin values did not differ very much from the previous year's values except in one or two cases. The nicotinic acid values were, however, either higher or lower than those of the last year's values, except in one or two cases.

160. Nutrition Research Unit under Dr. S.K. Gokhale at the Seth G.S. Medical College, Bombay.

I. NUTRITIVE VALUE OF PURE BRED STRAINS OF CEREALS

(a) *Successive years' harvests samples.*—In the year under report results have been presented for some of the selected strains of rice for two successive years' (1955-56 and 1956-57) crops for their content of the vitamins, riboflavin, nicotinic acid and folic acid and five essential amino acids, *viz.* valine, isoleucine, lysine, methionine and histidine.

It has been observed that there does not seem to be any appreciable change in the nicotinic acid and folic-acid and riboflavin content, in the successive year's harvest samples. The results obtained for the content of the five essential amino acids examined also do not show any significant change in the successive year.

Samples for three successive years crops (1954, 1955 and 1956) of nine pure bred strains of wheat obtained from the Botanical sub-station,

Pusa, were examined for the vitamins, riboflavin, nicotinic acid and thiamine. The results show no significant change in the content of these vitamins.

(b) *Effect of different methods of cultivation.*—Six samples of different cultivation methods, viz. local, departmental, Japanese, and three combination of these for two pure bred paddy strains, viz. Patnai-23 and Latisal from Chinsurah, West Bengal, i.e. twelve samples, were examined for the vitamins, nicotinic acid, riboflavin, thiamine and folic acid and five essential amino acids, viz. valine, isoleucine, lysine, methionine and histidine. Samples from Karjat (Bombay State) Rice Experimental Station, of paddy strain 'Kolamba-42' by local, departmental and Japanese methods of cultivation were also examined. No conclusions could be drawn from this meagre data. It is hoped to get more samples for such comparative data, sufficient to find whether these new cultivation methods, especially the Japanese, have an effect on the nutritional factors of the grain produced along with the observed increased output in production.

II. NICOTINIC ACID METABOLISM : PYRIDINE NUCLEOTIDES IN BLOOD

(a) *Blood pyridine nucleotides (PN) in hæmopoisis.*—It has been shown by earlier workers that pyridine nucleotides (PN) undergo changes in liver and blood in fatty infiltration of liver and blood PN levels are in index of liver PN stores. During the course of our investigation on the PN metabolism in hepatosplenomegaly, higher values for blood PN were obtained. Since the liver disease is often associated with anæmia, it was thought desirable to study the blood PN levels in the isolated anæmia cases without severe liver dysfunction.

Higher blood nicotinic-acid values in anæmia have been reported in literature (Kochhar : *Ann. Bioch. Exp. Med.*, 1942, 4, p. 211 ; and Melnick *et al*, *Jour Biol. Chem.*, 1942, 136, p. 157). Since nicotinic acid is mainly present in the form of PN in the red blood cells and leucocytes, the PN content in the former assumes special importance in studies of nicotinic acid metabolism in anaemias.

In the report, data has been presented for normal blood PN levels per c.c. blood and per c.c. of packed red blood cells for seventy-five healthy Indian male subjects. Averages for seventeen subjects studied of hepatosplenomegaly are also given. Results obtained for untreated cases of anæmia, thirty-five of hypochromic and eight of hyperchromic showed lower levels for PN per c.c. of blood and higher levels for PN per c.c. of packed red blood cells than the average normal figures obtained.

Eight cases of previously treated (with B complex and/or iron therapy) hypochromic anæmia showed subnormal blood PN levels, suggesting a relationship between B complex and iron and blood PN levels.

Eight cases of vitamin B₁₂ deficiency and ten cases of iron-deficiency anæmias were followed at weekly intervals for the changes in blood

PN levels during hæmopoisis - 100 μ g. of B_{12} administered intramuscularly and orally per week depending upon the gastric acidity. In the case of iron deficiency 130 μ g of ferrous iron was administered orally. Results of these investigations, (three typical cases of each), are presented in the report. It was observed that B_{12} and iron depressed the PN blood levels to subnormal levels which further tended to return to normal during progressive hæmopoisis. Retarded hæmopoisis was, however, found to be accompanied by an increase in the blood PN levels. The possible correlation of the blood PN levels with hæmopoisis is discussed.

(b) *Blood pyridine nucleotides in anæmia with liver cirrhosis.*—The work of investigation on blood PN changes in hæmopoisis indicated a possibility that a rise in blood PN retarded during hæmopoisis may involve the disturbance of PN metabolism in liver rather than the non-utilization of PN by the bone marrow resulting in the accumulation of PN in blood. The association of fatty infiltration of liver and hepatomegaly and beneficial effect of liver preparations in anæmia suggest the involvement of liver disturbance in anæmia. It was thought worthwhile to see whether characteristic changes in blood PN observed in anæmia bear any relationship with liver-function tests during the course of improvement of the liver function.

Data has been presented for the liver cirrhosis cases studied in the year under report. They received packed red blood cells transfusions at weekly intervals. The results so far obtained indicate the preference to hæmoglobin synthesis over plasma protein regeneration. The blood PN follows the usual pattern as in anæmia. An increase in blood PN is again seen in retarded hæmopoisis. Albumin, low initially, rose very slowly as compared to globulin which showed an increase initially.

III. PLASMA IRON AND COPPER

This investigation begun late in 1956, was not continued after December 1956 as per recommendations of the Nutrition Advisory Committee that it should not be taken up at that stage. A few more normal human subjects and twenty anæmia (iron deficiency) subjects were studied before the investigation was stopped.

161. Inquiry on intracellular distribution of vitamins under Dr. J. Ganguly at the Indian Institute of Science, Bangalore.

A. *Vitamin A esterase and cholesterol esterase.*—We have demonstrated that vitamin A esterase is different from esterase, because acetone treatment destroys the former but not the latter. Vitamin A esterase loses its activity more rapidly on aging or with increasing temperature, also several inhibitors preferentially inhibit its action.

The liver vitamin A esterase has no hydrolytic action on the vitamin A ester stored in the liver and it is proposed that a small amount of vitamin A ester is carried by blood from the liver and is hydrolysed by other tissues, such as pancreas, small intestine, etc.

Cholesterol esterase of rat liver was compared with the vitamin A esterase of the same tissue. Certain properties were common to both, such as both were present in the microsomal fraction of rat-liver homogenate and in the nuclear and microsomal fractions of chicken-liver homogenate. Both had similar optimum substrate concentrations and both lost activity on acetone treatment. Various inhibitors also had similar effects on both the enzymes. Both act on short chain fatty acid esters only and the Michaelis constants for both were nearly the same.

The points of difference between the two activities are: the pH optima for vitamin A esterase were at pH 6.6 and at pH 8.6, whereas the optimum for cholesterol esterase was at pH 7.4. The former activity was 10 to 12 times faster than the latter under optimal conditions of temperature, pH, substrate and enzyme concentrations. Cholesterol esterase was relatively more stable towards differential inactivation, such as aging and heat inactivation. The energies of activation were also different, for vitamin A esterase, 11,530 cal/mole. and for cholesterol esterase, 15,450 cal/mole. It would thus appear that the two activities are not identical.

B. Storage of vitamin A within the liver cell.—By blocking the reticulo-endothelial system of rats we have demonstrated that vitamin A ester is stored in the Kupffer cells and vitamin A alcohol in the parenchymal cells. That there is a difference in the mechanism of storage of vitamin A ester and vitamin A alcohol has been confirmed by further work, as described below.

Normal male stock rats were bled to death and the residual blood in the liver was perfused out with physiological saline. The livers were homogenized in 0.25 M sucrose solution and the supernatant fractions obtained therefrom after centrifuging off the nuclei, mitochondria and microsomes, when treated with various protein precipitants, yielded all the vitamin A in the precipitate. Ammonium sulphate at 50 per cent saturation precipitated almost all the vitamin A from the supernatant and the precipitate could easily be re-dissolved in distilled water. Treatment of the supernatant with various protein-denaturing agents or with trypsin released all the vitamin A, but that with sodium salicylate or ethanol at room temperature showed a difference in the relative release of the two forms of the vitamin. A difference in the distribution of the free and esterified forms of vitamin A was observed in the precipitates obtained on dialysing the supernatant against precooled buffers of varying pH values. Freezing and thawing released the whole vitamin A and cold ethanol at 18 per cent concentration precipitated the entire amount of vitamin A along with 23 per cent proteins. The precipitate could easily be re-dissolved in cold buffer. Electrophoresis of the supernatant on agar gel showed that vitamin A moves along with a lipo-protein band under electrical field. From these observations, we have concluded that vitamin A ester and vitamin A alcohol are associated with different lipoproteins within the liver cell.

It is noticed that after increasing doses of large amounts of vitamin A, the ester form of the vitamin in the liver progressively increases,

whereas the alcohol form, after reaching certain concentration does not increase any more. It is possible that the lipo-protein combining with vitamin A alcohol has limited ability to bind the vitamin A alcohol and as such gets saturated and does not bind any more of it, whereas in case of the ester the lipo-protein concerned may have wider capacity to bind the ester. This might explain the difference in the behaviour of the two forms of vitamin A in the liver during storage.

162. Inquiry on metabolism of starvation and malnutrition under Dr. M.L. Chakraborty at the Medical College, Calcutta.

From a study of 120 cases of malnutrition (human subjects) and (eleven) guinea-pigs in whom malnutrition had been produced experimentally, and 6 albino rats subjected to gradual starvation it can be inferred that there is a fall in the serum levels of sodium, calcium and magnesium. The fall in the level of calcium and magnesium has been very much insignificant and is very slow to occur. The serum potassium attains a high normal value. The total output of neutral urinary 17 ketosteroids, measured in 68 human subjects, 11 guinea-pigs and 6 rats (all were subjects of malnutrition) was very low. Thus, the low normal value of serum sodium and high normal value of potassium together with low output of neutral 17 ketosteroids suggest hypo-function of the adrenal cortex. X-ray of femur revealed osteoporesis. Examination of urine showed a greater excretion of calcium. These two observations suggest bony depletion of calcium, in conditions of malnutrition. Simultaneous with the fall in the levels of calcium, sodium and magnesium, there are also diminutions of blood sugar, cholesterol, urea and N.P.N. A few healthy cases under similar conditions were investigated to show the contrast with the cases of malnutrition with regard to blood picture, which will give an idea as to how the comparative deficiency of haematological and biochemical items are responsible for the production of altered clinical picture.

163. Inquiry on growth and Physical development of Indian infants and children under Prof. D.N. Mazumdar, Head of the Department of Anthropology, Lucknow University, Lucknow.

In conformity with the sample design framed last year, we started field work in the Central Zone of Uttar Pradesh, comprising of 1. Kanpur 2. Fatehpur 3. Allahabad 4. Lucknow 5. Unnao 6. Rae Bareilly 7. Sitapur 8. Hardoi 9. Fyzabad 10. Sultanpur 11. Partabgarh 12. Bara Banki. The following five districts were selected at random : 1. Kanpur 2. Allahabad 3. Lucknow 4. Sitapur 5. Partabgarh.

Besides a total of 898 cases have been studied by the ladies team in Kanpur, both for rural and urban areas which could not be tabulated, because they have just come in. The men's team after finishing work in the Central Zone moved into the Eastern Zone and started work in Benares where they collected data on 700 individuals which also could not be tabulated at short notice.

Last year a major part of our time was taken up in the standardisation of the technique of measurements. Herein, we have discussed various location of differences which can arise due to (a) same observations at different times (b) several observations by the same team at the same time (c) between teams working at different places. It was observed that our teams have attained a considerable degree of uniformity in measurements.

The 10th, 50th and 90th percentile points have been given for rural and urban areas as for males and females separately for each age group. In the case of girls, these percentile points have not been calculated for rural areas for ages beyond 12 years as the number of cases falling in these age groups were very small.

At the Seminar which was held by Indian Council of Medical Research at Lucknow from 2nd September to 12th September 1957, certain changes in the sampling frame work as well as in the presentation of data were suggested for further work. Statistical analysis has been kept waiting till all data are collected from the different centres in India. As a result of this change, further analysis was stopped. Henceforth, the data will be analysed on the pattern suggested by the Working Party.

164. Inquiry on relation of dietary fat and cholesterol to serum lipids under Dr. N. G. Magar at the Institute of Science, Bombay.

Animal experiments.—Forty-eight young rats, 4-week old and weighing between 40 g. and 50 g. were evenly distributed into four groups. Each group of rats was kept on a fat-free diet for a week. Afterwards the rats were maintained on diets containing twenty per cent of coco-nut oil, ground-nut oil, Vanaspati and ghee. 0.5 g. of cholesterol per kilogram body-weight of the rat was included in all the diets and the rats maintained on this diet for about 4 to 12 months. Rats were sacrificed at the age of five and eleven months from each group and total cholesterol, ester cholesterol, total phospholipids lecithin, sphingomyelin, cephalin, total fatty acids, lecithin, linoleic, linolenic, arachidonic pentanoic and hexanoic acids were estimated in various tissues and blood serum. Haematological studies were also carried out.

Forty-eight young rats are being maintained on a diet with five per cent level of fat—Vanaspati ghee, ground-nut and coco-nut oils. Rats were weighed twice a week and their daily food intake and growth recorded. Rats from each group will be sacrificed periodically and the analysis of tissues and blood will be carried as described above.

Human studies :

1. Thirty-two samples from healthy persons having their ages between 30 and 40, 40 and 50, and 50 and 60 were analysed for their total and ester cholesterol, total phospholipids, lecithin, sphingomyelin, cephalin. The different age groups were divided in sub-divisions depending upon their incomes. Their dietary habits were noted with

special references to animal and vegetable fat intake and foodstuffs rich in cholesterol.

2. Total fatty acids and linoleic, lonolenic, arachodonic, pentænoic and hexænoic acids were estimated in twenty normal human sera. Their age, income and dietary history were recorded.

165. Inquiry on the role of nutritional deficiencies in the causation of peptic ulcer under Dr. C. Raghavachari at the Stanley Medical College, Madras.

Introduction.—About 208 cases of peptic ulcer have been studied intensively from the diet survey, clinical and biochemical aspects. The methods followed have been standardised and the personnel fully trained and the results being obtained are found statistically significant. Of these 208 cases, 68 have been operated upon.

Among these 208 cases 68 are proved cases. Among these 60 cases (44 per cent are deficient in protein, 96.6 per cent in vitamin A₁; 11.7 per cent in vitamin B₁, 96.8 per cent in vitamin C, and 12.6 per cent in niacin.

These proved cases when compared with controls seem to be slightly deficient in vitamin C, vitamin B and vitamin A.

Diet survey by the "weighing method" were done in two of the patients houses. Their diet was also compared with diet of members of the same socio-economic group. It was found that these patients take lots of chillies and cloves. In both these families spices such as cloves etc., are used four times a week. In one family pepper is taken almost every day and in another 3 times per week.

These patients' diets when compared with controls, do not show much difference in the intake of protein. In this case the quality of is higher and the quantity normal. The diet consists of both animal and vegetable protein; whereas patients diet consists mainly of vegetable protein.

The survey was continued at the Medical College Hospital, Trivandrum. It is made on 27 patients suspected of peptic ulcer.

Cooked rice and tapioca from the staple food of these patients and these diets are deficient in calcium. Condiments, such as chillies and pepper, are added in large quantities in their diet. Though all of them take fish daily the quantity taken is very low. Vitamin B₁ derived from the diet of these patients are lower than the amount of B₁ derived from the diet of the patients in Madras.

Bio-chemical analysis of patient's blood.—Serum protein, serum albumin, serum globulin, electrophoretic pattern of blood proteins, serum lipids and iodine number and fractional test meal analysis in addition to vitamin C estimation have been done. The results obtained have been uniform and there are found significant deficiencies in ulcer patients as compared to controls.

Statistical survey of recurrent ulcer.—The period from January 1951 to June 1955 was selected for this purpose. The survey of all cases of recurrent ulcer admitted in Government General Hospital, Madras, has been completed with details regarding socio-economic as well as clinical aspects.

166. Studies on the effect of heat on the nutritive value of edible oils under Dr. R. Rajagopalan at the Indian Institute of Science, Bangalore.

It was found earlier that heated mustard oil neither depressed the growth of rats nor increased liver fat. When the experiment was repeated with another sample of oil, it produced the same type of adverse effects as observed with heated ground-nut, sesame and coco-nut oils. The factors responsible for the earlier result will be investigated. Heated butter fat also behaved exactly in a similar manner as the other heated oils. Practically, no counteraction of the adverse effects of heated oil was observed when the heated oil was mixed with an equal quantity of unheated oil and fed to rats.

Incorporation of methionine and cystine to the basal diet containing heated ground-nut oil gave complete protection to the rats. Growth with methionine was even better than the control, the liver fat also being lower. Cystine did not improve the growth in the same manner as methionine, but counteracted the growth inhibition and fatty infiltration of liver. With choline, growth inhibition was not overcome, but infiltration of fat into the liver was prevented.

The extent of destruction of vitamins added to diets containing heated oil was studied. The results showed clearly that thiamine, pantothenic acid, pyridoxine and choline were destroyed to varying extents. No destruction of niacin and riboflavin was observed. Vitamin A was destroyed completely in less than six hours when incorporated to the diet containing heated oil. In the case of rats receiving heated oil, the absorption, utilization and storage of vitamin A were lower than those in the corresponding control rats. Also, the conversion and utilization of β -carotene were decreased in rats receiving the heated oil. Thus, the results indicate the adverse influence of heated oils on vitamin A metabolism.

Studies on the digestibility of heated oil showed that the digestibility was not very considerably hampered due to heating.

The utilization of calcium, though less with heated oil, could not also account for all the adverse effects observed in growth experiments.

167. Inquiry into the effect of insect infestation on the nutritive value of foodgrains under Dr. M. Swaminathan at the Central Food Technological Research Institute, Mysore.

The objects of this investigation are as follows :

- I. Development of simple methods for the assessment of the degree of damage and the unhygienic conditions in food grains infested by insects,

II. Studies on the effect of insect infestation on the chemical composition and nutritive value of various food grains.

I. *Assessment of the degree of infestation and unhygienic conditions in infested food grains.*—No suitable method is available at present for assessing the unhygienic conditions in food grains caused by insect infestation. The only available method is that of counting the insect fragments in the milled products. This method is tedious and unreliable. There is therefore, a need for developing simple methods for assessing insect damage in grains and flours. Preliminary work from this Institute by Subrahmanyam *et al.* revealed that as insect infestation of wheat progressed, there was also a rise in the concentration of uric acid in the infested material. Uric acid is an important constituent of the excreta of insects and can be easily estimated by a simple colorimetric method. It was, therefore, decided to investigate whether the uric acid content of different grains infested by different insects, can serve as an index of the degree of damage and unhygienic conditions in the infested grain.

(a) *Studies on commercial samples of infested Bengal gram and field bean.*—Infested Bengal gram samples damaged to different extents were available in the local market and so work on these was taken up first. It was found that in insect infested Bengal gram, the uric-acid content increased with the increase in kernel damage. The increase, however, was not proportional to the kernel damage in certain cases. In Bengal gram (*Cicer arietinum*) damaged by *Bruchus* sp. damaged kernels, usually had 1 to 3 exit holes and in field bean (*Dolichos lablab*) 1 to 6 holes, each hole representing development of one insect in the kernel. A relationship between the number of insect exit holes and uric-acid content, therefore, appeared more likely. Further experiments carried out with infested Bengal gram and also damaged field bean showed that the uric-acid content of the infested samples is better correlated with the number of insect exit holes rather than with kernel damage.

(b) *Studies on cereals.*—Clean uninfested jowar was obtained within a month after harvest and stored in 12 B-twill jute bags (1'×1') each containing 5lb. of the grain. Five pairs of *Calandra oryzae* were initially let in each of the 6 bags. The rest of the bags were kept as controls in an insect-proof room. At the end of every month one bag from the control and the infested jowar, respectively, fumigated with ethylene dibromide (3lb./1,000 c. ft.) and observations on the physical condition of the grain, kernel damage, insect population, viability, weight/volume ratio, etc., were taken. Uric acid in the control as well as the infested sample was also estimated. A good correlation was observed between insect numbers, kernel damage and uric-acid content of the infested grain. There was a significant decrease in the weight/volume ratio and also in the viability of the grain with the continued progress of infestation.

(c) *Studies on wheat flour.*—Three 20-lb. samples of clean, uninfested 75% extracted flour (maida) in 25-lb. capacity tins were infested under controlled conditions with *Tribolium castaneum*; 100 insects were let in initially. The fourth 20-lb. sample was kept as control. The infestation was allowed to proceed for six months. 2-lb. samples

were drawn at the end of each month. The samples were passed through a 50-mesh sieve to remove the insects from the flour. The insect count (both adults and larvæ) was determined separately. The sieved samples along with the control were fumigated with ethylene dibromide (3lb./1,000 c. ft). A gradual increase in the uric-acid content of the flour samples was observed as the infestation progressed. The insect count of flour did not give any idea of the *degree* of infestation, whereas the uric acid went on steadily increasing in all the samples. No uric acid could be detected in the uninfested control samples. Even if infested flour was fumigated and cleaned of insects and sold in the market, the uric-acid content could very well serve as a good index of the degree of unhygienic condition in the material caused by insect infestation.

II. Investigations on the effect of insect infestation on the chemical composition and nutritive value of various food grains are in progress.

168. Goitre Pilot Survey Project (Pb.) under Dr. J.C. Ohri, Officer-in-charge, Goitre Pilot Survey Project, Pathankot.

The Goitre Pilot Survey Project was started on 18th November 1954 with headquarters at Dharamsala in Kangra District. It was initially entrusted with the task of surveying goitre endemicity in certain zones in Kangra District with the object of comparison of the prophylactic value of iodized and iodated salt as a goitre control measure.

Experimental zones.—Three zones were formed which were called 'A', 'B' and 'C'. Zone 'A' consists of 21 villages around Kangra town with an aggregate population of 37,858, zone 'B' of 30 villages around Nagrota with a population of 37,596 and zone 'C' of 20 villages around Baijnath and Palampur with a population of 37,328.

Goitre and hæmoglobin surveys were carried out in the three zones from November, 1954 to October, 1956 and, in addition, diet surveys were also undertaken. The areas were then placed under experiment by starting the distribution of iodized salt in zone 'A' and iodated salt in zone 'C' with zone 'B' serving as control where ordinary common salt is made available to the population. The distribution of iodized iodated salt in zones 'A' and 'C' was started from 19th December, 1956 and is continuing. Samples of iodised/iodated salt are being seized from zones 'A' and 'C' to assess the iodine content.

Goitre survey in Gurdaspur District.—The headquarters of the Project were shifted to Gurdaspur on 18-11-1956 with the object of starting goitre survey work in this District. Later, when it was found that goitre endemicity was high in Pathankot Tehsil, the headquarters were shifted to Pathankot on 20-4-1957 with the object of intensifying goitre survey work in this Tehsil and its adjoining areas.

Side by side with goitre survey, hæmoglobin estimation of the blood of almost all the individuals is made with the object of determining

the association of anæmia and goitre. A check of the incidence of caries and fluorosis is also made simultaneously. Height and weight measurements of all the individuals are taken and blood pressure of as many adults as possible is recorded.

The figures of survey work from December, 1956 to November, 1957 are as under :-

<i>Month</i>	<i>Goitre Survey</i>	<i>Hæmoglobin Survey</i>
December, 1956.	1428	1932
January, 1957.	1270	982
February, 1957.	1245	1058
March, 1957.	1479	910
April, 1957.	1661	1192
May, 1957.	2935	2442
June, 1957.	3674	3217
July, 1957.	3493	2925
August, 1957.	2856	2574
September, 1957.	3310	3052
October, 1957.	3294	2944
November, 1957.	3515	3211
Total :	30160	26439

Records of Goitre endemicity in each village surveyed are maintained age and sex-wise and are incorporated in the Monthly Progress Report of this Project. It has been found that Goitre endemicity is very high in certain parts of Pathankot Tehsil which are commonly known as "KANDI" and "ANDHAR" areas. Rice and Maize (Jowar) are the staple food of the inhabitants of those areas which have got excellent rice cultivation fields. It has been found that Goitre incidence varies in these two areas from 30 to 90 per cent of the population in various villages with an average of about 40 per cent for the entire areas. Incidence of Caries and Fluorosis has a direct relationship with that of Goitre.

Goitre survey work in Nurpur Tehsil of Kangra District.—The work was extended to Nurpur Tehsil of Kangra District with effect from 17th July, 1957. It has been found that Goitre endemicity is fairly high in the whole of this Tehsil and particularly in an area called "ANOH" area which is situated in the heart of the Tehsil. In some villages cent per cent children of this area have been found to have Thyroid enlargement. There is a stream (Khud) running in the middle

of this area which has been called the "GILHARI KHUD" (Goitre Stream) since ancient times. Villages on the banks of this stream present the largest sizes of hard and nodular ('D' stage) Goitre.

Goitre survey work in Hoshiarpur District.—The survey operations have been extended to Dasuya Tehsil of Hoshiarpur District with effect from the 11th November, 1957. Those villages which lie along the parts of Nurpur Tehsil beyond river Beas have been taken up for survey in the first instance.

Biochemical work.—Samples of drinking water supplies are seized from as many of the villages surveyed as possible and are analysed at the Biochemical Laboratory of the Project which is maintained in Medical College, Amritsar. Gross deficiency of iodine is found in practically all the samples which corroborates the generally accepted theory about the deficient intake of Iodine as the dominating factor in the ætiology of Goitre.

169. Scheme on relationship between malnutrition and bladder stones under Dr. D.A. Anderson at the Evangeline Booth Hospital, Ahmednagar.

(1) A Clinical paper on 123 cases of bladder stone operated upon in the Evangeline Booth Hospital from 1949-57 is under preparation.

(2) An Epidemiological study of all cases of bladder stone in all Hospitals in the District of Ahmednagar (so far as co-operation is received) for the same period has also been commenced.

(3) A detailed examination of 7 cases of bladder stone operated during the year ending September 1957 has been made under the following headings :

- (a) Chemical examination of stone.
- (b) Histological examination of bladder wall.
- (c) Biochemical examination of the patients serum, in particular, calcium, phosphorus, vitamin A and B, carotene.
- (d) Urine examination.
- (e) Rapid nutritional survey.
- (f) Diet questionnaire.

(4) A group of boarding school boys at Ahmednagar has been examined to establish normal variations in blood chemistry in the District.

(5) Similar examinations have been carried out on 30 members of our nursing and technical staff of the hospital.

170. A comparative study of the mother's blood, milk and blood of malnourished infants, under Dr. M. Thangavelu at the Medical College, Trivandrum.

The work was designed with the object of making a comparative study of the protein metabolism in the blood of malnourished children and their mothers and also to see whether there was any correlation in the protein pattern of the blood and milk of mothers and the blood of the children.

Twenty-three cases of malnutrition have been studied since October, 1957. The mothers were generally poorly built, belonging to the age group 20 to 30 years. The average weight was 87 lb., the minimum and maximum being 71 lb. and 123 lb., respectively. The weights of the infants varied from 8½ lb. to 28 lb. having an average of 15½ lb. for an average age of 1 year 11 months with a variance of 10 months to 9 years. In almost all cases, the infants were suffering from Kwashiorkor. The average number of children per mother was four, the maximum number being 10.

Some data on the socio-economic aspects of the mothers were also collected. Most of the cases were from the working class. The family size varied from two to twelve members with an average of 6.2. The average family income was approximately Rs. 65/- per month varying from Rs. 7½ to Rs. 160 per month. The diet was substandard, rice and tapioca being the main articles of food. Milk was a very rare item in the diet. When available, only small quantities were consumed which was given to the diseased infant.

Samples of maternal serum (and milk in a few cases) and serum of infants were analysed for total protein by Micro-Kjeldhal method. The percentage fractions of the proteins were obtained by paper electrophoresis.

The average protein value of the maternal serum was found to be 8.48 g./100 c.c. varying from 5.41 g. to 11.13 g. The high protein value in a few cases could not be explained—repeated estimations confirmed the high values. The average protein content of the sera of the malnourished infants was 4.54 g./100 c.c. ranging from 3.06 g. to 6.81 g. Only five samples of milk were available the average protein content being 1.265 g./100 c.c. with a range of 0.781 g. to 2.56 g. A comparison of the total protein in the blood and milk of the mothers in these few cases did not reveal any confirmation relationship. It was interesting to note that the total protein content of the infant decreased in the order of the decrease in the total protein content of the mother's milk except in one case.

171. Investigation on the chemical constituents of blood in marasmic conditions of infants and children under Dr. R. K. Dutta Chaudhuri at the Chittaranjan Cancer Hospital, Calcutta.

MATERIAL AND METHOD.

All the material under review came from Chittaranjan Sishu Sadan (Children Hospital of the Chittaranjan Seva Sadan College), Calcutta.

Four millilitres of venous blood was drawn in fasting condition ; serum calcium, serum phosphorus, serum protein (different fractions), total cholesterol, cholesterol ester, serum sodium and potassium were estimated according to various techniques.

At the very onset of the work micromodifications of the standard methods were done by examining 25 samples of blood from young doctors and laboratory personnels by both procedures to facilitate work with small samples of blood from infants. The results were as follows :—

Methods	Calcium, mg. per cent.	Phosphorus, mg. per cent.	Total cholesterol, mg. per cent	Cholesterol ester. mg. per cent
As stated above	11.16 ± 0.59	3.5 ± 0.48	157.0 ± 20.7	83.0 ± 9.13
Modification.	11.06 ± 0.67	3.4 ± 0.38	158.0 ± 21.7	84.0 ± 9.23

These results treated by 't' test showed that there was no significant difference between the two methods.

Selection of cases.—It is difficult to define clinically the marasmic condition in infants and children. Clinical criteria, such as loss of body-weight, loss of subcutaneous fat at different parts of the body, are not beyond criticism. But as quoted by Moncrieff and Evans (*Dis. Chil. I. p. 273*) loss of 35 per cent of expected body-weight can be considered as a criterion of marasmus. The defect of this method lies in great variation in normal expected weight of the infants. The expected weight of infants and children has been calculated by multiplying the average birth weight by 2 at 6 months of age, by 3 at 1 year of age, by 4 at 2 years of age and 5 at 3 years of age. The average birth weight of normal full term child was taken to be 6 lb. But loss of elasticity of the skin, loss of subcutaneous fat from abdomen, gluteal region, and buccal region were also considered along with it. The infants aged 4 months to 3 years were included in the present study. Premature babies and infants with slight oedema were excluded. But 3 cases of kwashiorkor were included when they reached true marasmic condition with the disappearance of oedema.

Chemical features.—Twenty cases have been studied (5 cases with loss of fat from all parts of the body, including succorial pad and with 55 per cent to 65 per cent weight loss, 12 cases who had lost subcutaneous fat from abdomen and gluteal regions only and with loss of 45 per cent to 55 per cent of the expected body-weight, 3 cases of kwashiorkor when they had reached true marasmic condition). The liver was not palpable in 7 cases of marasmus but all the 3 cases of kwashiorkor mentioned above had enlarged liver ; signs of vitamin 'A' deficiency were present in those 3 cases of kwashiorkor. Spleen was palpable in 2 cases and they were found to be suffering from tuberculosis.

Biochemical findings.—The total serum proteins of 16 marasmic infants varied from 5.0 mg. to 6.0 mg. per cent among the rest four cases, the 3 kwashiorkor ones had 3.75 g., 4.0 g., 4.2 g. per cent and the other one had 4.6 g. per cent. Albumin fraction was low in all the cases (1.2 to 3.0 g. per cent). Beta globulin was high (1.2 and 1.48 g. per cent) in 2 cases and they were found to be suffering from tuberculosis. Serum gamma globulin varied from 1.05 g. per cent to 1.76 g. per cent in 17 cases and 2.39 and 2.04 g. per cent in 2 cases and 0.75 g. per cent in 1 case, but it was a 4 months old child. These findings are in corroboration with Patel *et. al.* (*Ind. Jour. Child Health*, 6 p. 289, 1957).

Serum calcium varied from 9.16 mg. per cent to 10.33 mg. per cent in 18 cases, 2 cases showed low serum calcium, 8.6 mg. per cent and 8.8 mg. per cent, respectively. These 2 cases showed irregular prominent costo-chondral junctions in contradistinction to the other cases where costo-chondral junctions were prominent but smooth.

Serum phosphorus varied from 4.01 mg. per cent to 5.3 mg. per cent in 18 cases and in 2 cases (where calcium was also low) the serum phosphorus was 3.53 mg. per cent and 3.97 mg. per cent, respectively.

Total cholesterol varied from 115 mg. per cent to 145 mg. per cent in 15 cases, and 80 mg. per cent to 95 mg. per cent in 3 cases of kwashiorkor under-going marasmus.

Cholesterol ester varied from 58 mg. per cent to 95 mg. per cent in 15 cases, and 40 mg. per cent to 55 mg. per cent in kwashiorkor ones.

Serum sodium values varied from 125 milli equivalent per litre to 145 milli equivalent per litre in 7 cases.

Serum potassium varied from 4.8 milli equivalent per litre to 5.6 milli equivalent per litre in 7 cases.

FINDINGS ON COLLATERAL STUDIES DONE

1. The hæmoglobin values varied between 5.1 g. and 10.2 g. per cent.
2. Mantoux test (1:1000)—positive in 2 cases.
3. Infection : respiratory, 3 cases ; gastro-enteritis, 6 cases, bacillary dysentery, 4 cases ; urinary infection, 3 cases.
4. Infestations : giardiasis, 9 cases ; ascariasis in 5 cases.
5. Kahn test-negative in all the cases.

172. Inquiry on influence of soil fertility and genetic make-up on the yield and nutritive value of Indian pulses under Dr. G.C. Esh and Dr. U.P. Basu at the Bengal Immunity Research Institute. Calcutta.

Eighty-two pure-bred samples of various pulses, such as—Bengal gram, green gram, black gram, red gram, lentil, peas, horse gram,

khesari as grown in different parts of the country have been received upto now. Fifty of them have been chemically analysed for their protein, fat, calcium, phosphorus and crude fibre. The results collected so far tend to indicate that not only the different varieties of pulses vary in protein content (17 to 30 per cent) a great deal of variation in the protein content (18 to 28 per cent) of the same variety has been observed when raised at different localities. It is difficult to conclude at this stage whether such variation is due to genetic make-up and/or to soil conditions, unless the same strain pulse is raised in different localities. In case of Bengal gram when various strains are raised in the same locality a difference of 25 to 30 per cent in protein content has been observed.

173. Inquiry on separation of different forms of vitamin A₂ and effect of replacement of vitamin A by vitamin A₂ on small land animals under Dr. P.D. Dalvi at the S.M.S. Medical College, Jaipur.

The usual commercial sources which supplied fresh water fish liver oil concentrates with appreciable proportion of vitamin A₂ could not supply even a small quantity of the material. Also, the research workers formerly engaged in vitamin A₂ studies and a Government Department of the U.S.A., expected to be of use, were unable to help in this connection.

Some samples of fish liver oils from Indian sources were tried of these, one showed a gross E, 1% cm (328 m μ) of 0.66 and contained a fair proportion of vitamin A₂ as judged on the basis of the SbCl₃ colour values at 617 m μ and 693m μ . A few gallons of this oil is ordered for concentration either in a molecular distillation still or by saponification and later for chromatographic isolation of vitamin A₂.

A few experiments were conducted to prepare anhydro-vitamin A by a new method suggested by Shantz. In this method a benzene solution of vitamin A concentrate of high extinction value is re-fluxed with a requisite amount of p. toluene sulphonic acid. After treatment the cooled benzene solution containing anhydro-vitamin A is chromatographed to remove the weakly adsorbed anhydrovitamin A. Experiments with saponified vitamin A concentrates containing about 1 million i.u./8 resulted in small yields of anhydrovitamin A and with pure crystalline vitamin A gave a solution with inflexions at 360 and 375 m μ . The latter result is interesting in view of the work off Shantz on rehydrovitamin A with absorption maxima at 351 and 369 m μ .

Necessary spectroscopic solvents were prepared by conventional methods. The modern method of passing requisite pure solvents through 30 to 120 mesh silica gel did not work. The cause of the failure to obtain expected results has to wait till more work is done.

174. Inquiry on physiological studies on human milk and its role in infant feeding under Dr. A.C. Majumdar and Dr. Mrs. Amala Chaudhuri at the Institute of Health, Calcutta.

The inquiry deals with the analysis of random samples of milk from lactating mothers. The collection of milk samples was made

before and after feeding of the infant between 9 a.m. and 10 a.m. since this period, according to workers in the field, gives the maximum value for the various constituents of milk. Ten c.c. amounts were collected both before and after feeding; the samples were then pooled and analysed.

With the object of carrying out complete and detailed analysis, on small amounts of milk, micro-analytical methods practicable for laboratory use were adopted. All samples for analysis of the different constituents were transferred to a temperature of 15.6°C. within 20 minutes of collection, except in the case of specific gravity determination which was done at room temperature, the value at 15.6°C. being determined by the addition of 0.1 for each degree Fahrenheit above the temperature stated.

The following analyses were carried out by the methods given which were standardised :—

- (i) Specific gravity by the specific gravity bottle.
- (ii) Total solids and ash—gravimetrically. The ash was kept in 50 per cent Nitric Acid for analysis of the inorganic elements.
- (iii) Lactose by the micro-photometric methods (Folin and Wu).
- (iv) Fat by Babcock's method.
- (v) Total protein, albumin, casein and N.P.N. by the micro-digestion, direct nesslerization and photometry (Koch and McMeekin).
- (vi) Inorganic phosphate by the photometric method (Fiske and Subbarow).
- (vii) Inorganic chloride by the photometric method (Sendroy).

The mean, maximum and minimum values for the following eleven constituents on 19 random samples of human milk carried out at 15.6°C. are given below :—

	Max.	Min.	Mean
(1) Specific gravity	1036.1	1024.4	1031.5
(2) Total solids (W g/V c.c.) per cent	13.7	9.92	11.92
(3) Ash	0.3	0.128	0.174
(4) Inorganic chloride	0.200	0.100	0.1417
(5) Inorganic phosphate	0.010	0.0027	0.0059
(6) Fat	6.0	1.6	4.2
(7) Lactose	8.37	6.37	7.2
(8) Total protein	1.702	0.843	1.26
(9) N.P.N.	0.578	0.144	0.364
(10) Al etc.	1.27	0.589	0.925
(11) Casein	0.488	0.182	0.36

Complete histories of the mothers from whom milk samples were collected were taken, including nutritional and family history, past illnesses, present complaints and obstetric history.

**175. Inquiry on the mineral nutrition of lactic bacteria under
Dr. D. B. Desai at the B.J. Medical College, Poona.**

In the literature several references have been made to the antagonism of Na and NH_4 to K in respect of growth of certain bacteria and possibilities of improving the microbiological-assay methods using those bacteria, have been suggested by using media containing either no inhibitory ion at all or only a minimum amount of it. A systematic study of the factors to elucidate the nature of antagonism of Na and NH_4 to K was considered worthwhile from the point of view of improving ultimately the microbiological-assay methods for vitamins and amino acids for which the lactic bacteria have been commonly used.

Investigations carried out so far have been with the medium and the procedure of Henderson and Snell (*Jour. Biol. Chem.*, **172**, 15, 1948), modified to exclude all K or Na from the medium as and when necessary. Pure acetic acid and phosphoric acid were used and the medium was neutralized by KOH, NaOH or by NH_4OH as required. *L. arabinosus* 17-5 and *L. mesenteroides*-P-60 were the organisms studied.

Following features of the effect of Na, K and NH_4 on the growth of these organisms were revealed

1. Na is definitely capable of promoting growth even when present alone in the medium. The growth-promoting effect of K is much more than that of Na. NH_4 ion is, however, quite incapable in this respect, when present alone in the medium.

2. The growth, whether due to Na or K, is different for the two organisms studied for any particular concentration of the ion. It is more marked for *L. arabinosus* than for *L. mesenteroides*. This suggests that the mineral-needs differ from bacteria to bacteria.

3. When the medium contains both Na and K, increasing concentration of Na is found to inhibit the growth due to K at lower concentration of the ion but to stimulate the growth at higher concentrations of it. However, only up to a certain concentration of Na, this stimulatory effect is observed and beyond it, again the inhibitory effect.

4. NH_4 acts antagonistically to Na and even at low concentration inhibits completely the growth due to Na. With increasing amount of Na, the amount of NH_4 bringing about complete inhibition of growth is also found to be increasing.

5. The effect of NH_4 on the growth due to K is different from the effect on the growth due to Na. At low concentrations of NH_4 stimulatory effect is observed but at higher concentrations, inhibition. The concentration of NH_4 completely inhibiting the growth is also very high when K is present. As in the case of Na, the amount of NH_4 bringing about complete inhibition of growth is also increased with increasing concentrations of K up to a certain concentration. Whatever may be the concentration of K beyond this limit, the amount of NH_4 completely inhibiting the growth appears to be the same.

6. With the increased buffer capacity of the medium by increased acetate and phosphate, increased growth was obtained. The replacement of acetate by citrate in the medium had no effect on the growth.

Future plan of the work :—

1. To investigate the cause of difference in the requirements of the bacteria for the mineral ions particularly Na, K and NH_4 —whether it is due to certain vitamins or amino acids.

2. The work will then be extended to one or two pathogenic bacteria.

3. It is also proposed to study the effect of Na, K and NH_4 ions on glycolysis by resting cells of the bacteria.

It is believed that the work may not go beyond the estimated period of two years as mentioned in my original proposal.

Reference : (1) Henderson L. M., and E. E. Snell, *J. Biol. Chem.*, **172**, 15, 1948.

176. Inquiry on malignant malnutrition in children under Dr. L.S.N. Prasad and Dr. H. Saran at the P.W. Medical College, Patna.

A comparative study is being made of the effect of mixed protein diet and multi-purpose-food, supplied by the Central Food Technological Research Institute, Mysore, in cases of malignant malnutrition in children. The few cases so far investigated showed a low blood protein percentage, which gradually rose to normal level during the course of the treatment. The weights of the children 'on admission' were 30 to 50 per cent of the expected weight. The maximum weight gained by a child has been 4lb. during one month. Bowel infection, anæmia, etc., if present, are being suitably treated. The cases are being followed up.

177. Study of articles of food, the diet and dietary habits of various tribes of N.E.F. Agency under Dr. S.R.K. Iyengar at the Health Training & Research Centre, Pasighat.

In Mirem, a tribal village in Pasighat Sub-division, a diet survey was conducted in May, 1957. Mirem is a recent settlement in the plains by the tribals who migrated after great Assam earth-quake in 1950. There are 74 families with a population of 373. The villagers are all Abors belonging to the class Minyong.

Survey and methods.—Seven families with a population of 30 were studied for one week using schedules recommended by All India Institute of Hygiene and Public Health, Calcutta, and Nutrition Advisory Committee.

Dietary habits.—They usually take two principal meals in a day and a small lunch in the field kept out of the morning meal. As a rule, their food does not contain cooking oil, condiments, spices, milk products

sugar, jaggery and fruits except occasionally jack-fruit and oranges. They take eggs, meat and fish when available. Hunting is the main source of animal proteins. On festive occasions they sacrifice Mithun (*Bos frontalis*), pigs, fowls, goat, etc. They preserve the excess of meat by smoking. They take hand pound rice and they do not wash or throw away the conjee (surplus liquor), while cooking rice. Majority of the people take a home brewed 'Apong' rice beer, which has been analysed by some workers and found to be nutritive.

The survey showed that there was both qualitative and quantitative inadequacy of diet.

Calories.—The average per capita was 2,200 per adult. This is too poor when compared to the amount of manual labour men and women do.

Proteins.—Animal protein was practically absent during the survey. The daily consumption of 60 g. of proteins were mainly from vegetable source which is of a low biological value.

Fat.—Animal fat was particularly absent—the daily consumption of 9 g. was from vegetable fat.

Carbohydrate.—This was deficient though it was the main source of energy.

Vitamins.—Both fat and water soluble vitamins were insufficient.

Mineral salts.—Calcium and phosphorus were inadequate.

Nutritional status.—Average height of adult was 5 feet with an average weight of 100 lb. The following evidence of malnutrition was found :

(i) Angular stomatitis 59 per cent, (ii) glossitis 41 per cent (iii) caries tooth in 38 per cent, (iv) bleeding gums 23 per cent (v) loss of adipose tissue 55 per cent, and (vi) few cases of xerosis of conjunctiva.

Study of the articles of diet.—A large series of vegetables of local origin have been sent to Regional Botanist, Botanical Survey of India, Shillong, for identification.

A large samples of water has been collected for estimation of iodine, fluorine, calcium and other mineral contents.

Study of goitre, cretinism and deafmutism.—Incidence of goitre, cretinism and deafmutism in majority of the surrounding villages (15) has been surveyed. In some of them incidence of goitre is as high as 60 per cent with 4 to 5 per cent cretinism and deafmutism.

CLINICAL & FIELD TRIALS WITH PROTEIN RICH FOODS

178. Inquiry on field trials of protein rich foods under Dr. C.N. Rukmini, Child Welfare Scheme, Corporation of Madras, Madras.

Two different slum areas which are having Child Welfare Centres of Corporation of Madras were selected. The centres' buildings are made use of for the collection of children and distribution of the food with the kind permission of Corporation of Madras. Eighty cases were initially enrolled in each centre (1-5 years) and clinical examination and diet survey done. Height and weight were taken.

Mysore Food A was distributed in Chetpet and Mysore Food B in Choolai—both in ball forms, each ball containing roughly 7.5 g. of protein.

Mixing ratio

I. Mysore A	...	5 lb.
Jaggery	...	1 lb.
Water	...	q.s. to ball
II. Mysore B	...	5 lb.
Jaggery	...	26 ozs.
Water	...	q.s. to ball

These were distributed as follows once a day :

1-2 years	...	1½ balls	11.25 g. of protein
2-3 years	...	2 balls	15.00 g. of protein
3-4 years	...	3 balls	22.50 g. of protein

Attendance in Chetpet

May	...	64 children
June	...	60 children

Attendance in Choolai

May	...	50 children
June	...	36 children

From July 1st, it was decided to try two more foods. So, the children of different streets were divided into two different groups in both the centres. In Choolai some children from old roll were taken and rest were newly enrolled after initial survey. In Chetpet altogether new set of children were selected for the new food trial. In both the centres children of 6 months to 1 year were also enrolled.

Foods distributed as follows :

CHOO LAI	{	Forenoon	...	Bengal gram, skim milk mixture
		Afternoon	...	Mysore B
CHETPET	{	Forenoon	...	Skimmed milk
		Afternoon	...	Mysore A

Note.—Since the children were not liking ball form and invariably they were requiring water to drink while taking the balls, it was decided to give both Mysore foods in the form of cooked 'Payasam', each fluid oz. containing 2.5 g. of protein.

Mixing ratio :

Mysore A	...	Jaggery=3 : 1	} (still bitter)
Mosore B	...	Jaggery=4 : 3	
Skim milk	...	Jaggery=4 : 1	

Bengal gram : Skim milk : Jaggery = 4 : 1 : 1

Bengal gram, skim milk mixture is given in the form of ball, each ball containing 7.5 g. of protein.

Each food is distributed to supply protein as follows :

		Grammes
6 months to 1 year	...	7.50
1 year to 2 years	...	11.25
2 years to 3 years	...	15.00
3 years to 5 years	...	22.50

Quantity of payasams and skim milk distributed :

(1 fl oz. = 2.5 g. of protein)

		Ounce
6 months to 1 year	...	3
1 year to 2 years	...	4½
2 years to 3 years	...	6
3 years to 5 years	...	9

Attendance :

CHOO LAI

	Mysore B	Bengal gram skim mill mixture
July	44	53
August	42	50
September	32	45

Note.—Number of children for Mysore B went down because of the bitterness of the food even after adding more jaggery (non-acceptability)

CHETPET

	Mysore A	Skimmed Milk
July	65	47
August	60	35
September	50	23

Note.—Number of children for skimmed milk went down because of the complaint of diarrhoea in about 50 per cent of cases.

Follow-up

1. Height and weight taken once a month.
5. Clinical examination once in 3 months.

Biochemical examination.—Serum protein estimation started in September 1957 in the Biochemistry laboratory of the Pediatric Department, Government Hospital, Madras. So far 3 cases have been done.

179. Investigations on clinical trials with protein rich foods under Dr. R.N. Chaudhuri at the School of Tropical Medicine, Calcutta.

The work was started recently after a course of training of the Assistant Research Officer at the Nutrition Research Laboratories, Coonoor. So far, only 9 children, aged 2 years to 4 years, with signs of protein malnutrition were subjected to clinical trial. Of these, 4 were cases of nutritional marasmus and 5 kwashiorkor. The *chief clinical features* were evidence of failure of growth, general emaciation, with or without œdema, diarrhoea in most and fever of a low irregular type in some.

The *dietetic history* revealed that they were getting very little proteins and calories. Careful history taking, physical examination and investigations to eliminate infections and infestations were made in each case. All cases were thus of nutritional deficiency.

For the trial, Mysore mixture B, skimmed milk, and Mysore mixture A were used in this order. No preference of food was given to any case, in relation to clinical features. The diet contained 40 g. to 60 g. of protein from the vegetable (Mysore) food or skimmed milk powder, according to the age/or ability of the child to take nourishment. The rest consisted of sugar with or without bread so that the total calorie was 1,300 to 1,600.

Although it was planned to give 60 g. of protein (i.e. 140 g. of Mysore mixture A, 154 g. of Mysore mixture B and 176 g. of skimmed milk) without discrimination of age, but in practice, it was not possible as all children could not consume the whole amount. Actually, the diet contained 40 g. to 60 g. depending on the age and ability to consume it. All the children included in this series of nine cases took the diet fairly well for one month, when they were discharged from the hospital. Three

children had diarrhœa at the time of admission, but the diarrhœa was controlled with diet alone. Another one had frequent abdominal distension in the earlier part but later tolerated the diet well.

All the children in this small series improved with the diet, the improvement being more marked with skimmed milk than with the vegetable protein (Mysore mixtures). A case of kwashiorkor with usual skin and hair changes, could not tolerate 'Mysore B' diet. She developed diarrhœa within two days and the diet had to be discontinued. Another girl refused to take 'Mysore B' as she disliked the taste. A third child, a case of kwashiorkor, died on the third day of 'Mysore B' diet. These three cases were of course excluded from the series.

180. Inquiry on clinical trials of protein rich foods under Dr. S.T. Achar, Director, Institute of Pediatrics, Government General Hospital, Madras.

Selection of cases.—Out of the cases attending the 'Kwashiorkor' Clinic in the Pediatric Out-patient Department Government General Hospital, Madras, selected cases were admitted for hospitalisation and trial of protein rich foods. They were admitted into this Ward and given the following protein rich foods.

Selection of foods.—The following protein-rich foods were tried.

1. Animal protein in the form of skim milk.
2. Pure vegetable protein in the form of Mysore Food A.
3. Combination of vegetable and animal protein in the form of a mixture of Bengal gram and skim milk.

MATERIALS AND METHODS

Total number of cases admitted	...	34
Cases transferred to T.B. section	...	6
Cases discharged against medical advice	...	5
Cases died	...	4
Cases investigated	...	19

Foods were given as follows :

1. Skim milk 5 oz. reconstituted with 30 oz. of water.
2. Mysore Food A—4 oz. mixed with jaggery in the proportion of 2 : 1 boiled for 5 minutes in 30 oz. of water.

3. Bengal gram and skim milk and jaggery in the ratio of 4 : 1 : $\frac{1}{2}$ and a mixture of 6 oz. of this reconstituted with 30 oz. of water boiled for 5 minutes.

Out of the 19 cases investigated, 6 cases had skim milk, 8 cases Mysore Food A, and 5 cases the mixture of Bengal gram and skimmed milk.

Supplemental feeds.—In addition to what they get as above, they were given 6 oz. to 8 oz. bread and one orange per day and 2 oz. of sugar. Strict vigilance is kept day and night with the help of the Social Worker and Ayah that these patients do not get any other food supplements. Their ailments and gross vitamin deficiencies have been treated with suitable antibiotics and vitamin supplements.

Clinical Examination and Investigations.—These cases, when admitted, were subjected to clinical examination both on the day of admission and subsequently daily till their discharge and the findings recorded in a standardized *proforma* prepared for this purpose. Their weights were recorded daily in the morning before starting food. Heights were taken on admission and discharge.

Routine urine and motion examinations were done.

Blood was drawn for hæmatology and serum-protein estimations on admission and also at the time of discharge. Separation of serum protein by paper-electrophoresis method had been started and the instrument is stabilized.

Mantoux test done in all cases and where necessary x-ray chest was also taken.

Liver biopsy.—In addition to the above, liver biopsy was done both at the time of admission and discharge whenever possible. Total number of cases so far biopsied 10.

181. Inquiry on field trials with protein rich foods under Dr. Mrs. Muktha Sen, All India Institute of Hygiene and Public Health, Calcutta.

After a short period during which the staff were given opportunity to familiarize themselves with the area of work the base line survey was started. Till the end of December 1957, 267 children were examined by taking the height and weight and hæmoglobin. Stool was also brought for examination whenever possible. The results of the hæmoglobin examination in percentages and the stool examination is given below. It will be seen that even from this base line survey, some interesting information will be available for our follow-up. Plans are being made to complete the base line survey and start the feeding experiment in two areas.

HAEMOGLOBIN PERCENTAGE OF CHILDREN BLOOD

Per cent	Number
31—35	—
36—40	—
41—45	2
46—50	4
51—55	8
56—60	40
61—65	33
66—70	75
71—75	40
76—80	46
81—85	16
86—90	3
91—95	—
96—100	—

100 per cent = 14.5 grammes.

STOOL EXAMINATION OF CHILDREN

Total specimen = 105

Helminths (Ova)	Protozoa	Charcot Leyden crystals
<i>Ascaris lumbrici</i> 20	<i>Giardia</i> 18	3
<i>Hookworm</i> 1	<i>E. histolytica</i> 5	
<i>T. Trichura</i> 1	<i>E. coli</i> 7	
<i>H. nana</i> 1	<i>E. nana</i> 3	
<i>E. vermicularis</i> 1	<i>T. hominis</i> 1	
24	34	3

HUMAN LACTATION

182. Studies on human lactation under Dr. J.W. Airan at the Wilson College, Bombay.

During the period under report, leucine, phenyl-alanine, threonine, arginine, histidine, tyrosine and cystine were estimated in samples of human milk collected in Bombay.

In all, thirty samples were analysed. Out of these, fourteen came from three mothers, about three to four from each. The first sample in

these cases was collected on about the 10th day after delivery, as this could be easily done in the hospital. Subsequent samples from these sources were obtained at the interval of a week to cover a total period of about a month after delivery. Further samples were then collected at the end of the second month; the third month, and the fourth month. The remaining were random samples obtained on the 10th day when mothers were in the hospital.

The total nitrogen in the different samples of milk was found to vary considerably. Thus in, one case the minimum was 230 mg. and the maximum 390 mg. per 100 c. c., whereas in another case the minimum was 145 mg. and the maximum was 250 mg.

The amount of total amino acids were calculated to 100 mg. of nitrogen in the hydrolysate of each sample.

As read off from their graphs, lysine, threonine, leucine, tyrosine and cystine contents were found to be, on an average, maximum on the 20th day and arginine content was minimum near about the 25th day after the delivery. Phenyl-alanine was found to be maximum on the 50th day, when threonine, cystine histidine and lysine contents were beginning to drop down. Histidine however was found to show variations.

In order to get a comparative picture, the amounts of various amino-acids as read off from the graph for the 20th day after delivery are given below :—

Total amino-acids in mg. calculated to 100 mg. of N in the hydrolysate.

Case Number	Total N in milk mg./100 cc.	Leucine	Phenylalanine	Threonine	Arginine	Histidine	Lysine	Tyrosine	Cystine
1	240	102	34	42	61	118	78	45	21
2	165	102	32	51	68.5	108	80	46	10
3	230	87	24	47	68	106	82	40	12

The amounts of total amino acids in the 14 random samples collected on the 10th day of the delivery varied between the following limits, (mg./100 mg. of N in the hydrolysate) :—

Leucine	(70 to 90)	Phenyl-alanine	(20 to 30)
Threonine	(30 to 60)	Arginine	(50 to 70)
Histidine	(80 to 100)	Lysine	(50 to 70)
Tyrosine	(25 to 50)	Cystine	(10 to 15)

183. Studies on human lactation under Dr. C.V. Ramakrishnan at the Baroda University, Baroda.

Fifty milk samples from lactating mothers belonging to various socio-economic group and different lactation period have been analysed for essential amino acids, vitamins, such as B₁, B₂, B₁₂, folic acid, pantothenic acid, niacin, vitamin C, total protein, albumin globulin, lactose, fat and acidity. Data have also been collected regarding the general condition of health of the mothers and their children as well as their socio-economic status. No attempt has been made to derive any conclusions from the data obtained so far since large number of data will be necessary in each group for statistical analysis and interpretation of the data.

PROTEIN METABOLISM

184. Inquiry on protein metabolism in under-nourished children under Dr. R.N Chaudhuri at the School Tropical Medicine, Calcutta.

Detailed clinical studies of 33 under-nourished children aged from 9 months to 5 years were made. They included : (i) Marasmus with severe emaciation, no subcutaneous fat, and no oedema or skin changes—13 cases, and (ii) Kwashiorkor with (a) all the classical features—4 cases, and (b) oedema only—16 cases.

History revealed that the illness started about the age of 6 months in most of them following inadequacy of breast milk and supplementary feeding with barley or sago water and little milk with sugar, the diet being grossly deficient both in calories and protein. They all belonged to low economic status and the parents had several other issues. Five had *tuberculous* infection of the lungs. Four were infested with round worms and two with hookworm. All were anæmic and hypo-albuminæmic. Linear lines at the lower end of the radius, suggestive of arrest of bone growth, were radiologically demonstrated in 5 out of 14 cases studied.

Results.—Twenty-two patients responded well to skimmed milk. Ten cases came in a very low condition and died shortly afterwards. Another case also failed to respond and died after being treated for a month.

Post-mortem examination was done in 6 cases. Besides the findings, such as wasting, fatty liver, broncho-pneumonia and/or distended intestines, etc., in 5 consecutive cases an unusual histological picture of 'teratomatous changes' was observed in the myocardium.

Nitrogen balance studies done in two patients show that they could retain greater part of the nitrogen absorbed, though in one, infested with ascariasis the absorption was found to be considerably impaired.

185. Studies on calcium, phosphorus and protein metabolism including utilization of mixtures of dietary vegetable proteins under Dr. M. Swaminathan at the Central Food Technological Research Institute, Mysore.

The objects of the inquiry are as follows :

- (i) To study the metabolism of calcium, phosphorus and protein in children and adult human subjects on diets based on cereals and millets.
- (ii) To study the utilization of mixtures of dietary vegetable proteins by human beings.
- (iii) To study the role of cellulose and hemicellulose on the absorption of protein, calcium and phosphorus.

Determination of the chemical composition of ragi, raw milled rice and tur dhal.—Preliminary chemical analysis of the different foodstuffs required for the work has been carried out and the experimental techniques standardized. In addition to this the *in vitro* digestibility and availability of starch from cooked ragi and rice are also being studied using salivary and pancreatic amylases. Similar studies on the *in vitro* digestibility and availability of proteins from cooked ragi and rice are also being conducted with pepsin and trypsin.

Metabolism of nitrogen, calcium and phosphorus in children and adults on diets containing rice, ragi and tur dhal.—Studies on the effect of replacing 25 and 50 per cent of rice in poor rice diets by ragi on the metabolism of nitrogen, calcium and phosphorus in children and adults has been started. Growth and metabolism experiments on albino rats using diets having the same composition as those used in human metabolism studies are in progress with a view to find out the overall nutritive value of the diets.

186. Inquiry into the protein requirements in pregnancy and lactation under Dr. H. N. Banerji at the G. R. Medical College, Gwalior.

In the year under review the scope of the investigation was limited to nitrogen-balance studies in healthy pregnant women in the second and third trimesters of pregnancy. Accordingly, twelve healthy pregnant women who were initially checked up by the obstetrician to eliminate any abnormality and at intervals thereafter, were hospitalized for nitrogen-balance studies. They were put on 60 g., 90 g., 120 g. levels of proteins in diets containing 2400 ± 200 calories with two multivitamin tablets daily. Twenty five to fifty per cent of the proteins were of animal origin, such as milk proteins. All the subjects were vegetarians.

Subjects were put on the scheduled diets for seven days for adaptation, followed by a three-day period on the same diet for the collection of urine and faeces for 3 consecutive days for analysis of nitrogen excretion.

Subjects varied in age between 16 and 40 years, height 58 and 60.5 inches, weight from 39 kg. to 70.3 kg. and in parity from primipara to ninth para. All the subjects except two, were available for study on only one level of protein intake for 10 days only. Of the two exceptions one was studied on all three levels of protein intake and the other one on only two levels, 60 g. and 90 g. in the third trimester of pregnancy.

Diets were mainly composed of cereals, such as rice and wheat, pulses (red gram and green gram), vegetables leafy and non-leafy, milk, milk powder, casein, threptin biscuits, ground-nuts, fruits, oil, ghee (clarified butter), sugar, condiments and occasionally tea or coffee. The diets were made by very careful weighing, cooked without any loss and consumed completely by the subjects.

24-hour samples of urine and faeces were collected very carefully over a few drops of toluene for three consecutive days and analysed for nitrogen. Creatinine excretion in urine was checked up daily during the period of collection. Replicate samples of food were analysed for nitrogen, fats and carbohydrates by difference. Health Bulletin No. 23 was also very frequently used for compilation of diets.

All the subjects have shown negative nitrogen balance varying between 0.003 g. and 1.53 g. on 60 g. level of protein intake. Progressive nitrogen retention has been shown by the subjects when diets contain 68.75 g., 90 g., 98 g. and 120 g. of proteins.

187. Inquiry on protein requirements (a) in infancy and childhood; (b) in adults; and (c) pregnancy, under Professor M.P. John at the P.W. Medical College, Patna.

This work has been undertaken in order to find out the minimum amounts of protein required at different periods of pregnancy. Two cases have been investigated at 20th and 14th week of pregnancy. Diets

containing 11.28 g., 14.81 g., and 24.34 g. nitrogen were administered consecutively to each patient, the woman at 20th week of pregnancy showed nitrogen retention per day of 5.35 g., 8.65 g., and 16.57 g. respectively, and the woman at 14th week of pregnancy showed nitrogen retention per day of 4.24 g., 8.06 g., and 17.55 g., respectively.

ENERGY METABOLISM

188. Studies on energy metabolism under Dr. S. Banerjee at the Presidency College, Calcutta.

Studies on the determination of the basal metabolic rates of Indians.—Basal metabolic rates of 128 normal males and 85 females of different age groups were determined simultaneously by the open circuit (Douglas bag) method and by the use of Collin's Metabolex apparatus. The average R.Q. of both males and females measured by the open circuit method was 0.84. Most of the values of B.M.R. obtained by Collin's Metabolex apparatus were slightly lower than those obtained by the Douglas bag method. The B.M.R. measured by the open circuit method was on the average 12 per cent lower than the Mayo Foundation standards in the case of females and 17 per cent in the case of males.

The mean B.M.R. of the males of ages varying from 13 to 15 years was 38.39 calories per hr./per sq. m. of body surface. The respective values for age groups between 16 and 17 years, 18 and 19 years and 20 and 24 years were 36.96, 34.75 and 34.64 calories per hr. per sq. m. of body surface.

The mean B.M.R. of the females of ages varying from 18 to 19 years was 32.25 calories per hr. per sq. m. of body surface and that of ages between 20 and 24 years was 31.64 calories per hr. per sq. m. of body surface.

Study on the relation of basal metabolic rate to body composition.—To find out whether or not the total cell solid or cell mass is a more suitable standard of reference than the surface area of the body for the expression of the basal metabolic rate, body compositions of 20 normal subjects (14 males and 6 females) were determined by the method of McCance and Widdowson. Total body water (urea space), thiocyanate space, extracellular fluid volume, (corrected thiocyanate space), serum volume, blood volume, interstitial fluid volume, cell solids, cell mass, body fat and minerals were determined. The age of the subjects varied from 17 to 39 years with an average of 23 years; the weight varied from 40.8 kg. to 69.8 kg. with an average of 51.3 kg. the height varied from 150.3 cm. to 176.7 cm. with an average of 163.1 cm.

The average serum volume and corrected blood volume were 2.3 and 4.0 litres respectively. The average total body water, volume of distribution of thiocyanate and extracellular fluid volume, interstitial fluid

volume, intracellular water of the subjects were, respectively, 31.7 ± 0.8 , 12.4 ± 0.5 , 11.6 ± 0.3 , 9.3 ± 0.2 and 20.1 ± 0.5 litres and respectively, formed 62.1 per cent, 24.3 per cent, 22.7 per cent 18.3 per cent and 39.4 per cent of the body-weight. The average cell solids cell mass, minerals and body fat were, respectively, 9.9 ± 0.3 , 30.0 ± 0.8 , 3.1 ± 0.1 and 6.8 ± 1.1 kg., and respectively, formed 19.4, 58.8, 6.1 and 12.3 per cent of the body-weight. The total body water expressed as percentage of lean body mass varied only between 70.0 and 72.1 per cent with an average of 70.8 per cent. All these values are comparable to those obtained by other workers.

Corelation coefficients between the total basal oxygen consumption and the different variables, e.g. cell solids, cell mass, lean body mass and surface area were calculated. As revealed by the 'Z' transformation test, the correlation coefficient between oxygen consumption and cell solids (+0.934) or cell mass (+0.935) was significantly higher than that between oxygen consumption and surface area (+0.715). The variability of oxygen consumption among the subjects was much less when it is expressed per kg. cell solids or cell mass than when it is expressed per sq. meter of body surface. All these suggest that cell solid or cell mass representing the active protoplasmic mass is a more suitable standard of reference than surface area for the expression of basal metabolism of persons living in tropical countries like India.

Comparison between the various methods for the easy determination of lean body mass.—Body fat of sixteen male and nine female subjects was determined by various methods, such as by the formula of Rathbun and Pace using the specific gravity of the body actually determined by the water displacement method, by the formula of Keys and Brozek using density of the body obtained by the water displacement method or by the method of McCance and Widdowson from the *in vivo* determination of extracellular fluid, cell mass and minerals. The average body fat obtained by the method of McCance and Widdowson was 12.3 per cent of the body weight. This is comparable to the average value of 11.0 per cent calculated from the actually measured specific gravity by the formula of Rathbun and Pace, and also to the average value of 11.3 per cent calculated by the formula suggested by Keys and Brozek.

Specific gravity of the body was again calculated from the different regression equations suggested by Brozek and Keys using different skinfold measurements at different sites, such as abdomen, chest, below scapula, over triceps, over the belly of biceps. Comparison of correlation coefficients of values of specific gravity calculated from different skinfold measurements and of that actually measured by the water-displacement method indicate that the specific gravity of the body calculated from the scapular skinfold measurement and also from the combination of skinfold measurements of abdomen, arm and chest had high correlation with the specific gravity of the body actually measured. Statistical test with 'Z'—transformation, however, revealed that there is no significant difference between the coefficients of correlation between actually measured specific gravity and that calculated from different formulæ using different skinfold measurements. So, any one of the formulæ

of Brozek and Keys may be used with equal accuracy. Therefore, different skinfold measurements may advantageously be used in place of actual measurements of the specific gravity of the body by the water-displacement method due to the easiness and readiness with which the skinfolds can be measured, and hence fairly accurate estimation of lean body mass of Indians can be made.

CARBOHYDRATE, VITAMIN & MINERAL METABOLISM

189. Metabolic studies in the B group of vitamins with special reference to folic acid and vitamin B₁₂ under Dr. A. Sreenivasan at the University of Bombay, Bombay.

1. *Glycine-serine interconversion*.—A study of the *in vitro* synthesis of serine from glycine by liver enzymes using various 1-C precursors was completed. Among the additions tried were formate, methionine, methanol, histidine, betaine and sarcosine. Large differences in the effects of the various additions obviously reflect differences in rates at which the 1-C fragment is donated by them for the glycine to serine conversion. Sarcosine aids serine formation maximally. The methyl group of sarcosine is derived exclusively from the β -C of serine, while the carboxyl and α -C atoms of serine form their counterparts in the glycine moiety of sarcosine (*Jour. Biol. Chem.*, **186**, 361, 1950). The high values for biosynthesized serine even without added glycine would point to an oxidative rearrangement of sarcosine through sarcosine oxidase (*Jour. Biol. Chem.*, **133**, 211, 1944). The methyl group of methionine is utilized only to a limited extent (*cf. Jour. Biol. Chem.*, **186**, 275, 1950; **214**, 821, 1955).

2. *Glycine-choline interconversion*.—The participation of folic acid in glycine to choline conversion is also demonstrable from observations in blood and liver levels of choline in animals reared on an 8 per cent casein diet with 1 per cent glycine supplementation. Other determinations included liver and blood levels of nitrogen, methionine, neutral and phospholipids and total sulphhydryl.

That in addition to the known influences of folic acid in glycine to serine conversion, the formation of choline from serine is also influenced by the vitamin is seen from the results of experiments with serine supplementation in place of glycine. An important observation was the enhanced synthesis of choline in the groups supplemented with cystine, thus suggesting that utilization of methionine probably precedes choline synthesis (*cf. Jour. Biol. Chem.*, **190** p. 31, 1951; *Biochem. Jour.*, **55** p. 270, 1953).

Studies on methionine oxidation and on tissue phosphoryl choline and glycine synthesis are in progress.

3. *Nitrogen retention in relation to dietary fat levels.*—Substitution of fat for carbohydrates is known to result in decreased nitrogen retention (*Jour. Nutr.*, **56**, p. 139, 1955). Using a peanut protein diet at 18 per cent level with 4 and 10 percent supplements of sesame oil, preliminary indications have been obtained pointing to a favourable effect of folic acid on nitrogen retention as well as on liver lipids.

4. *Choline and methionine biosynthesis in Neurospora.*—The ability of respiring cells of *N. crassa* (wild) to synthesize methionine from homocysteine was examined of various I-C donors studied, formate and serine were found to be the most effective. Studies on the incorporation of choline into phospholipids with the wild and the cholineless mutant of *N. crassa* revealed that as the choline level in the incubation mixture was raised incorporation into the mycelium reached a maximum and then decreased.

5. *Vitamin B₁₂ and oxidative metabolism.*—The work was continued on the following lines.

(a) *Carbon tetrachloride injury and liver stores of vitamin B₁₂.*—The observations would suggest that the steatogen acts by provoking a temporary release of the vitamin from cellular particles to plasma (cf: *Proc. Soc. Exper. Biol. Med.*, **94** p. 497, 1957).

(b) *Absorption of vitamin B₁₂ in thyrotoxicosis.*—Contrary to the reported obstruction in vitamin B₁₂ absorption in chicks fed iodinated casein, it was observed that in rats, there was no interference in the absorption of injected vitamin B₁₂ from the gut which appeared to be solely determined by the extent of vitamin B₁₂ deficiency in the animal.

(c) *Thyrotoxicosis and changes in activity of certain phosphatases.*—The ATPase activity of hyperthyroid animals was increased by nearly 60 per cent, the major increase being limited to mitochondrial fraction. Pyrophosphatase and acid phosphatase also showed increased activities, while alkaline phosphatase activity was slightly reduced. Vitamin B₁₂ counteracts these changes to a considerable extent. It is known that increased ATPase activity of mitochondria is an indication of its structural damage (*Jour. Biol. Chem.*, **191**, 485, 1951).

(d) *Pyridine-nucleotides.*—In view of the major importance of pyridine nucleotides on phosphate uptake during electron transport to molecular oxygen, studies were carried out on (i) the effect of vitamin B₁₂ deficiency on *in vitro* incorporation of administered nicotinamide into liver PN (cf: *Jour. Biol. Chem.*, **219**, p. 287, 1956), and (ii) changes in liver PN in carbon tetrachloride poisoned animals. The results (*Nature*, in press) bring out the importance of vitamin B₁₂ in regulating liver PN and PN/PNH.

(e) *Sulphydryl metabolism.*—In hyperthyroid animals, there is observed an enhancement in glutathione synthesis with vitamin B₁₂ protected animals. Similar results were observed in experiments with carbon tetrachloride poisoned animals.

Thus, the lowering of blood and liver vitamin B₁₂ levels in the hyperthyroid as well as in CCl₄ administered animals is closely paralleled by depletion of glutathione reserves of these tissues. Correspondingly, the incorporation of administered cysteine into liver and blood glutathione also suffered. There is also a damage to cellular morphology. It is possible that prior administration of vitamin B₁₂ exerts protection against damage to cell structure through conservation of sulphhydryl which might play an important part in maintenance of mito-chondrial structure and permeability (*cf: Jour. Biol. Chem.*, **222**, p. 341, 1955).

Work is being continued on the possible participation of rat liver nuclei in glutathione biosynthesis. The existence in rat blood erythrocytes of an enzyme system capable of conjugating the constituent amino acids to form glutathione has been shown. The system is being investigated.

190. Inquiry entitled intestinal synthesis of B-complex vitamins under Dr. R. Rajagoplan at the Indian Institute of Science, Bangalore.

In the present experiment, the effect of curds on the intestinal synthesis of vitamin B₁₂ has been studied in rats. The rats received a basal diet containing 18 per cent protein (casein) and adequate quantities of all the vitamins. Vitamin B₁₂ intake in all the groups was 0.05 µg/rat/day. Group I served as the control. Group II received curds supplement, Group III received sulphaguanidine supplement and Group IV received curds and sulphaguanidine supplements. The data on growth, show that the group receiving curds has a better growth whereas the group receiving sulphaguanidine is as good as the control. The group receiving curds and sulphaguanidine compares with that receiving curds only. As a result of ingestion of curds, the urinary and faecal excretion of vitamin B₁₂ as also the liver stores have been increased. On the basis of the above results, it may be stated that feeding curds along with the basal diet definitely leads to increased intestinal synthesis of vitamin B₁₂ and that the vitamin so synthesized is absorbed, utilized and stored in the liver.

In another experiment, the intestinal synthesis of riboflavin as influenced by feeding curds from fresh milk, skimmed fresh milk, whole milk powder (KLIM), and skimmed KLIM was studied. The data relating to growth, show that all the experimental groups have improved growth, more or less, to the same extent and better than the control group. The results on urinary and faecal excretions as also the liver stores show curds from fresh milk, skimmed fresh milk, KLIM and skimmed KLIM enhance the intestinal synthesis of riboflavin in rats.

191. Studies on metabolism of vitamin K and riboflavin under Dr. S.C. Roy at the University College of Science & Technology, Calcutta.

I. (a) *Further purification of the prothrombin preparation.*—Prothrombin prepared from ox blood (*Record Chem. Prog.*, **13**, 143, 1952)

with an activity of 1,000 to 1,200 units per mg. was further purified to 1,600 to 1,700 units per mg. by improving the working condition and standardization of the process. Briefly, this consisted of diluting oxalated bovine plasma 16-fold and acidifying to pH 5.1. The precipitate was dissolved in oxalated saline and the prothrombin was adsorbed on magnesium hydroxide from which it was eluted by decomposing the hydroxide with carbon dioxide. The eluate was fractionated with saturated ammonium sulphate, followed by dialysis and isoelectric precipitation at pH 4.6. This prothrombin was redissolved in water and inactive materials were removed by treatment with barium carbonate. The final prothrombin solution was dried from frozen state with an activity of 1,600 to 1,700 units per mg. as determined by the method of Ware (*Amer. Jour. Clin. Path.*, **19** 471, 1949). Elementary composition : C—46.11 per cent ; H—6.77 per cent and N—13.85 per cent.

(b) *Electrophoretic studies of purified prothrombin.*—The above preparation was examined in Tiselius electrophoretic apparatus. There were 3 components, the main one consisting 90 per cent of the total protein with two other components. The pattern was similar to that as obtained by Seegers *et al.* (*Blood* **5**, p. 421, 1950).

(c) *Treatment of purified prothrombin preparation with Weber's solution and characterization of the fractions.*—The prothrombin preparation (1,600 units per mg.) on treatment with Weber's solution (0.6 M KCl, 0.04 M NaHCO₃ and 0.01 M Na₂CO₃) yielded fractions one of which after dialysis at 0°C. for 48 hour against 50 per cent saturated ammonium sulphate, showed vitamin K-like activity, while the residual fraction retained in cellophane bag was devoid of it. The dialyzate was dried, extracted with ether, ether evaporated off leaving a residue. This fraction is termed W-Pr A and those remained in cellophane bag was made salt-free, dried and termed W-Pr.

The W-Pr. A was dissolved in alcohol and responded to colour reaction characteristic of the naphthoquinone type of compounds (*Analyst*, **65**, p. 7, 1940). The W-Pr and ether-extracted residue did not respond to this reaction. Similar colour reaction was obtained with synthetic vitamin K preparation, 'Synkavit' (2-methyl-1,4-naphthohydroquinone disphosphate, Na-salt).

These fractions were tested for vitamin K-activity biologically, using albino rats. The W-Pr A was active and found to reduce the coagulation time of deficient rats from 58 to 27 sec. and the reduction was from 58 to 24 sec. with 'Synkavit'. The W-Pr and whole prothrombin were inactive biologically.

The W-Pr A was found to be a mixture of micro-crystals and brown oil. From the mixture the crystals were separated from a mixture of acetone and petroleum ether (1:1) and purified by re-crystallization from a mixture of chloroform and methyl alcohol (1:1). The fraction was further purified over zinc carbonate column. The crystals had absorption maxima at 225 and 294 m μ , while the oil had maxima at 228 and 258 m μ , when measured in the Beckman UV spectrophotometer.

They had respective minima at 252 and 250 m μ . Elementary composition: C-65.23 per cent; H-5.64 per cent; and N-nil. The infrared spectrum of the oil fraction was obtained having peaks at 2.85, 3.43, 5.81, 5.84 and 7.3 μ and the corresponding characteristic groups are as follows : OH, -CH(phenyl ring), C=O, O-COCH₃ and side CH₃.

The infrared study of the crystalline fraction is under way.

(d) *Determination of N-terminal amino acids of prothrombin preparation.*—As a preliminary to the identification and estimation of the N-terminal amino acids in prothrombin, 2:4-dinitrophenyl derivatives of the following amino acids have been prepared according to the method of Sanger (*Biochem. Jour.*, **39**, p. 507, 1945): Glycine, threonine, aspartic acid, valine, proline, phenylalanine, isoleucine, α -alanine, histidine, glutamic acid, cystine, lysine, arginine and methionine.

II. METABOLIC SYNTHESIS OF RIBOFLAVIN AND ITS ANALOGUE.

(a) *Utilization of nitrogen from different sources.*—Study of the effect of single nitrogen source in the form of inorganic ion or amino acid, purines, pyrimidine on the formation of riboflavin by the bacteria has been made. It has been observed that amongst other nitrogenous compounds urea stimulates the synthesis of riboflavin to the extent of 6 μ g./ml. of the broth.

(b) Chromatographic studies of the broth indicate that certain intermediary and analogous compounds are formed during the synthesis of riboflavin,

192. Studies in carbohydrate, vitamin and mineral metabolism under Prof. M.C. Nath at the University Department of Biochemistry, Nagpur.

(1) The effect of glucose-cyclo-acetoacetate, a substance formed by condensing glucose and ethyl acetoacetate on the cure of alloxan diabetes has been studied.

Rabbits, which were made diabetic, by the administration of alloxan, were used for experimental purpose. Experiment was started on animals showing more than 300 mg. blood sugar/100 c.c. after the fifth day of alloxan injection (intravenous). Sodium salt of glucose-cyclo-acetoacetate was given in 2 different proportions (140 and 280 mg./kg., respectively) intramuscularly, in daily doses for eight weeks and the change of blood sugar and urine sugar levels were studied.

(2) The glucose-cyclo-acetoacetate, on acid hydrolysis was found to yield a dienol form of glucose. To isolate and identify this glucose, paper chromatographic studies were undertaken. Circular paper chromatogram was done, using butanol-acetic acid water mixture (50 : 10 : 40). A special technique involving the use of 10 per cent phosphomolibdic acid and 10 per cent NH₄NO₃ was found to yield good results in developing the chromatographs. Dienol compounds develop

blue colour with this special technique, Vitamin C and hydrolysed glucose-cyclo-acetoacetate extracted and purified, were utilized, for preparing the chromatograms.

(3) The effect of glucose-cyclo-acetoacetate (which has been reported from this Laboratory to be present in normal blood) on the growth rate of normal animals was studied. For this study rats were chosen. To the normal diet of the experimental animals, was included 0.5 per cent by weight of the diet, glucose-cyclo-acetoacetate, prepared and purified in our Laboratory. The feeding experiments were continued for 5 weeks. The increase in the body-weight was recorded to be higher in the case of glucose-cyclo-acetoacetate fed animals, than in case of the normal animals.

(4) The study on the effect of glucose-cyclo-acetoacetate on the prevention of experimental liver necrosis, was undertaken. Preliminary works on the prevention of experimental liver necrosis caused by the injection of carbon tetrachloride, 2.0 c.c./kg. body-weight, by the hydrolysed product of glucose-cyclo-acetoacetate, have been completed.

193. Studies in carbohydrate, vitamin and mineral metabolism under Dr. S. Banerjee at the Presidency College, Calcutta.

Studies on carbohydrate metabolism in scorbutic guinea pigs.—Scurvy is associated with disturbance in carbohydrate metabolism. In scurvy diminished glucose tolerance has been reported in many species of animals. Banerjee and Ghosh observed a decreased liver and muscle hexokinase activity in scurvy. The decreased turnover rate of phosphorylated intermediates of carbohydrate metabolism has been observed in scurvy. Banerjee has also observed degenerative changes in the islets of Langerhans and diminished insulin content of pancreas in scurvy.

There are evidences that insulin is involved in the intermediate metabolism of carbohydrates at the level of Kreb's cycle. In the present work effect of injection of insulin on the glucose-tolerance test and liver and muscle glycogen contents in scorbutic guinea-pigs have been studied. As a preliminary study, the tissue contents of alphaketo-acids, citric, malic, and lactic acid of normal, scorbutic and insulin-treated scorbutic guinea-pigs have been estimated.

Scorbutic guinea-pigs showed lowered glucose tolerance. After insulin treatment glucose tolerance showed considerable lowering of blood sugar 150 and 180 minutes after glucose feeding although it still remained significantly higher than that of normal. Insulin treatment also brought about the shifting of peak of blood sugar value from 150th to 90th minute period after feeding glucose.

Glycogen content of liver and muscle diminished in scorbutic guinea-pigs which was restored by treatment with insulin. This indicates that prolonged insulin treatment can correct to a great extent the disturbed carbohydrate metabolism in scurvy.

It is reported that diabetes is associated with an altered pyruvate metabolism with resultant high blood pyruvate level. Since, scurvy simulates diabetes in many aspects of metabolism of carbohydrate, it was surmised that scurvy may also be associated with an alteration in pyruvate metabolism. Except in blood, no significant change in the concentration of alpha-keto acids in tissues of scorbutic guinea-pigs was observed. Possibly, scurvy is not associated with any derangement in metabolism of keto-acids.

An increased content of citric acid in tissues of scorbutic animals was observed which was brought to normal level by treatment of insulin. This defect in citric-acid metabolism may be either due to an accelerated rate of its synthesis or to a metabolic block below the level of citric acid in the Kreb's cycle. Insulin corrected this defect which indicates that insulin, in some way, facilitates citrate metabolism in scurvy possibly by activating the aconitase enzyme.

STUDIES ON NUTRITIVE VALUE OF FOODSTUFFS

194. Inquiry on the nutritive value of foodstuffs under Dr. M.V. Radhakrishna Rao at the Haffkine Institute, Bombay.

The preliminary work on the inquiry has already been started and a sample of maize-tender (*Zea mays*), divided into four lots, was analysed separately, for twenty three different nutrients, on the lines indicated in the Report of the Working Party (1956), appointed by the I.C.M.R., in this connection.

195. Studies on nutritive value of foodstuffs under Dr. M. Srinivasan at the Central Food Technological Research Institute, Mysore.

With the object of making the Health Bulletin No. 23 (fifth edition) complete with respect to the data and availability of essential constituents in natural and processed foods, the following lines of work were suggested :

- (a) Filling up the gaps in the fifth edition of the Health Bulletin No. 23.
- (b) Extending the analysis of the foodstuffs listed in the above Bulletin, firstly for phytin phosphorus for vegetable foodstuffs, pyridoxine, pantothenic acid, choline and vitamin B₁₂ in animal foods, and later on for sodium, potassium, magnesium, copper, sulphur, oxalic acid, chlorides, acid-base balance, inositol, biotin, para-amino-benzoic acid, ionisable iron and iodine.

- (c) Work on uncommon foodstuffs of regional importance : collection of information on their availability and mode of consumption; analysis of these foods for the constituents mentioned in (b) in addition to those listed in the Health Bulletin No. 23.
- (d) The amino-acid composition and the biological value of proteins in foods.
- (e) The effect of cooking on the nutritive value.

The work was started with the first phase, namely of filling up the gaps in the fifth edition of the Health Bulletin No. 23. Out of two hundred and seventy seven materials in which gaps exist, forty were analysed for the constituents, such as fibre, carotene, thiamine, nicotinic acid, riboflavin and ascorbic acid.

196. Studies on nutritive values of foodstuffs under Dr. B.C. Guha at the University College of Science and Technology, Calcutta.

As suggested by the Working Party on Nutritive Values of Foodstuffs, the work was concentrated to fill up the gaps existing in Health Bulletin No. 23. The following foodstuffs have been analysed for some nutrients which had not been estimated before and had not appeared in the Bulletin : cholam (*Sorghum vulgare*), maize, dry (*Zea Mays*), Sak alu, drumstick (*Moringa oleifera*), rose apple (*Syzygium jambos*), lichi, mango ripe (*Mangifera indica*), red gram (*Cajanus cajan*), lentil (*Lens culinaris Medic*) black gram (*Phaseolus mungo*), Khesari (*Lathyrus sativus*), jack fruit ripe (*Artocarpus heterophyllus*), green gram (*Phaseolus aureus* Roxb), pine apple (*Ananas comosus*) and wheat flour, atta (*Triticum aestivum*). These foodstuffs were also analysed for their copper, sulphur, chloride, sodium, potassium, magnesium, acid-base balance, oxalate, phytin phosphorus and available iron contents which are not included in the Health Bulletin. From the values obtained it was noted that samples from different localities varied considerably in their nutrient contents. Among the foodstuffs analysed maize has the highest amount of carotene, about 4600 $\mu\text{g}/100\text{ g}$. Samples of drumstick contained sulphur ranging from 284 mg. to 639 mg. per 100 g. which was highest among the foodstuffs analysed. Oxalic-acid content of drumstick was also highest about 100 mg/100 g. Jack fruit contains a considerable amount of riboflavin (about 85 $\mu\text{g}/100\text{ g}$.) besides carotene, the value for which is given in the Health Bulletin.

Besides the above-mentioned foodstuffs, a few samples of canned vegetables and fruits, *viz*, karela (*Momordica charantia*), parwar (*Trichosanthes dioica*), kankrol, pink beans (*Phaseolus vulgaris*), mango (pulp and slice) have also been analysed for their vitamin contents. It was observed that these canned food-stuffs contained considerable amount of the vitamins—B₁, C, riboflavin, niacin and carotene. Mango (pulp or slice) contained about 1350 μg of carotene and kankrol about 67 mg. of ascorbic acid per 100 g. after 2 years' storage. All these canned foodstuffs were found to contain riboflavin in the range of 53 to 80 $\mu\text{g}/100\text{ g}$. of the foodstuffs.

FOOD TECHNOLOGY

197. Investigations on parboiling of rice under the Director, Central Food Technological Research Institute, Mysore.

With a view to improving the quality of commercial parboiled rice in India and popularizing its use among all sections of rice-eaters, the working party on 'Food Technology' of the Nutrition Advisory Committee of the Indian Council of Medical Research initiated an inquiry on the subject of parboiling of rice in two centres in India. The following is the report of work done at the Central Food Technological Research Institute, Mysore.

The applicability of the principle of 'hot soaking' of paddy to reduce the soaking time and to eliminate the fermentation during soaking was tested on a factory scale in rice parboiling mills. After studying the conditions obtainable in rice mills in India, it was practicable and profitable to use the cement tanks at present used for soaking for steaming the paddy also. A suitable type of steam distributor was fitted to the tank to enable uniform parboiling of the paddy. The method was demonstrated on a large scale and rice millers have evinced keen interest in adopting this method.

Some operational data on the drying characteristics of steamed parboiled paddy were collected using a pilot plant scale mechanical drier, as a first step towards evolving a large-scale mechanical drier suitable for Indian conditions.

Studies were also conducted on the calcium enrichment of rice. The use of calcium-chloride solution for soaking of paddy during the parboiling process was found to increase the calcium content but was uneconomical for routine fortification. Calcium carbonate dusted on to parboiled rice was easily leached out by water washing prior to cooking. It was found that calcium carbonate at 85 per cent incorporation into wheat flour could be processed into a dough which can be pressed into vermicelli or rice shaped grains. This premix was found to stand washing with water and could be added to rice at a dilution of 1 : 250 to increase the calcium content of rice to about 100 mg. per cent.

198. Investigations on parboiling of rice under Dr. B.C. Guha and Dr. A.N. Bose at the University College of Science and Technology, Calcutta and the College of Engineering and Technology, Jadavpur, Calcutta

The work is sub-divided under two broad headings :

(1) Studies on the physical, chemical and biochemical properties of parboiled rice prepared on a laboratory scale under different conditions, and (2) Studies on the characteristics of parboiled rice prepared on a pilot scale.

(a) *Steeping of paddy.*—The effects of temperature and volume of water on the rate of soaking of paddy were investigated in detail. In the present series of experiment, the effects of short steeping time (not exceeding 6 hours) were studied. It was found that at 75°C. a period of 2½ hours may be sufficient for bringing the moisture content of steeped paddy up to 50 to 60 per cent, which is generally necessary for processing.

(b) *Drying of processed paddy.*—The drying rates of differently processed (steeped and steamed) paddy were determined in a laboratory oven at different temperatures (80°C., 90°C. and 100°C.) and the drying characteristics and the drying rates were observed. The drying rate in each case was found to be a 'falling rate'.

(c) *Changes of the vitamin B₁ content of parboiled rice.*—Detailed studies are now being carried out on the loss of vitamin B₁ content of rice during soaking, steaming and drying of paddy in the process of preparation of parboiled rice. It has been found that soaking at an elevated temperature (up to 75°C.) for a fairly short time (2 to 3 hours) does not reduce the vitamin B₁ content of paddy to a significant degree.

(d) *Digestibility of starch of parboiled rice.*—Changes of the digestibility of starch of parboiled rice prepared under different conditions of soaking, period of steaming and temperature of drying are now being studied. The digestibility experiments are carried out with taka diastase and β -amylase prepared in the laboratory from wheat.

(e) *Effect of pre-steaming in the preparation of parboiled rice.*—During the process of steeping of paddy, the enzymes present in rice bring about some reactions forming undesirable colours and to avoid it pre-steaming of paddy to destroy them might be desirable in some cases. The effect of this pre-steaming process on the soaking characteristics of paddy has been studied and it was observed that pre-steaming of dry paddy does not affect significantly the rate of soaking.

GROWTH & PHYSICAL DEVELOPMENT OF INDIAN INFANTS & CHILDREN

199. Studies on growth and physical development of Indian infants and children under Dr. M.V. Phadke at the Sassoon Hospital and B.J. Medical College, Poona.

The investigation is a part of an integrated study to be carried out simultaneously at some centres under the auspices of the Indian Council of Medical Research. At Lucknow, it was suggested during the Seminar that uniformity will soon be introduced in the staff working in various units and we were told that some additions to the present staff will therefore be made. Meanwhile we were asked to start the work with whatever equipment and staff available to us. The Statisti-

cian has accordingly collected all the data necessary for determination and distribution of sampling in the Poona area. The sample has now been decided upon and selection of children and actual fieldwork will start soon.

Meanwhile school authorities of various pre-primary, primary and secondary schools have been approached. The nature of the proposed research work, its significance and co-operation required from them was explained to them. From the discussions held it is felt that co-operation would be forthcoming when actual work is started.

The remaining Child Welfare Centres and Baby Clinics are also being approached. The Sassoon Hospital to which the Research Worker is attached also runs a Well Baby Clinic and has a maternity Ward of about 80 beds. It is, therefore, felt that infants and children of all ages and from various social strata will be available in numbers enough to cover the estimated sample. Field work has now started and over 1,000 observations have been made.

200. Studies on the growth and physical development of Indian infants and children under Dr. P.C. Biswas at the Department of Anthropology, University of Delhi, Delhi.

The Indian Council of Medical Research has undertaken studies on the growth and physical development of Indian infants and children as a project under the Second Five Year Plan. The object of these studies is to obtain correct and scientific information by well-planned studies on the growth patterns of Indian children and the various factors which influence them. Information so gathered will enable us at a later date to find out how far the changing conditions in India were affecting patterns of growth and physical development as determined in the coming years. Besides this, these studies have considerable scientific significance, because they will also help us to collect information on the various dietary, environmental and social factors which influence the growth of children.

Area of investigation.—The area to be covered by the unit comprises the states of Delhi, Rajasthan, Punjab and Jammu & Kashmir.

Preliminary work.—In order to eliminate personal errors among the field investigators, the standardization procedure was carried out between the two anthropologists, as regards techniques of measurement. The correlation between the measurements by the two anthropologists on the same subjects, as well as the correlation between the measurements on the same subjects by a single anthropologist at different times was studied.

Pilot survey.—A pilot study was then undertaken with the object of studying the field problems and difficulties that might arise during the main survey and for testing the schedule. The pilot survey was confined to the city of Delhi and the population for the survey consisted of children of age group 0-15 years belonging to different socio-economic classes in the city.

The pilot study has yielded some valuable information which has been useful in improving our methods during the main survey.

Following completion of the pilot survey, the main survey has been taken up after possible improvements in design as suggested by the conclusions of pilot survey. At present, school going children belonging to different age-group are being studied.

201. Inquiry into the somatotyping of male college students in Nagpur under Dr. J.N. Berry at the Medical College, Nagpur.

Somatotyping of one thousand male college students in Nagpur on lines of Sheldon *et al.* (1940) by the technique as adopted by Tanner *et al.* (1949) and Tanner (1951) is being done. Besides finding the basic physique patterns and measuring androgyny or femininity of build in the male and comparing these with the somatotypes in similar age group male college students in America as found by Sheldon *et al.* (1940) and in Oxford students as found by Tanner (1951), this work should form the frame work of later studies of distribution of physique patterns in different racial and social groups and their relationship to diseases, particularly cardio-vascular diseases or the change or otherwise of somatotype with age.

By including all the male college students in a class the sample should be fairly representative of male college students in Nagpur. All the male medical college students (500) will be somatotyped and then it will be extended to include students from other college or colleges.

A Cannon VT camera with 135 mm. lens with fine grain (27)^o film and photographs from 8 metre distance and enlargements to half plate size on glossy smooth paper will be made. Weight is taken by a lever type machine. The first 80 students have been photographed with Graflex Super D with 2¼" × 2¼" film adapter with 10" lens from a 9 metre distance to see if use of larger film justified the increased cost and the labour involved in repeated change of films, but the preliminary trials with 35 mm. Cannon which has just become available have given us good, if not better results.

So far, 80 students (all the male students in 1st year Medical College) have been photographed.

LATHYRISM

202. Investigation on lathyrism under Dr. (Mrs.) K.T. Ganapathy at the Gandhi Memorial Hospital, Rewa.

The objectives and purpose of the study are (a) to make a thorough clinical and biochemical study of established cases of lathyrism (b) to undertake field investigations so as to assess various factors connected

with the incidence of the disease, including dietary and nutritional background, (c) to spot early cases with a view to study the evaluation of the disease and to carry out therapeutic trials, (d) to collect specimens of lathyrus seeds from different localities and under different conditions of storage from the households of subjects suffering from lathyrism, and (e) to collect post-mortem materials with a view to study the histopathological changes in nervous system.

Regarding the 3rd objective of the working plan the unit has recently undertaken investigation of two cases which just complain of pain in the lumber region which radiates down the thighs with or without paræsthesia. With regard to the 5th objective the unit is watching constantly for obtaining suitable autopsy.

CLINICAL WORK IN LATHYRISM

Twentyseven established cases of lathyrism have been examined in the hospital for clinical and biochemical investigations.

Cranial nerves and sensory disturbances have not been detected in any of the cases. In very few cases reflexes in the upper extremities are brisk.

Abdominal reflexes are lost in most of the cases depending upon the level of lesion. In the lower extremities almost all cases show exaggerated knee and ankle reflexes. The planter is always extensor. Ankle clonus is very much marked.

Muscular power, specially in dorsiflexors of the ankle joints and abductors of thighs appear to be weakened, but it may be due to the spasm in the calf and hamstrings. In some cases of lathyrism coordination of movements in the lower extremities cannot be performed due to muscular spasm otherwise it is normal. No sensory loss of any type has been detected. Joint and Vibration sensations are perfectly normal, none of the cases complain of any bladder or sphincter trouble.

Rossclimos reflex, in which a blow on the ball of foot with the pateller hammer elicits flexion of the toes, and the Bechtere Mendel's reflex in which the same response follows a tap on the cuboid bone, are present in some of the cases.

Cerebrospinal fluid of the patients has been examined for sugar and chloride and this has been found to be within normal range. C.S.F. protein has been done in one case and found to be raised.

STUDY OF EARLY CASES

Two subjects have been examined for clinical and biochemical changes. Both the patients complain of pain in lumber region. The pain is constant from 2 to 3 years. It increases after heavy work and during rainy season.

FIELD STUDY OF LATHYRISM

Diet survey.—Diet survey in 61 families of lathyrism patients in Satna and Rewa Districts have been carried out. The period of survey was extended to seven days. Some of the heads of families are small cultivators, but most of them are landless agricultural labourers. The principal crops of the area surveyed are wheat, Bengal gram, Khesari (*Lathyrus sativus*).

II. *Nutrition survey.*—Nutrition survey of 127 subjects was carried out in the families where diets have been surveyed and also on other subjects, including school children.

Deficiency diseases, which were seen during the survey are; Xerosis pigmentation of conjunctive, night blindness, phrynoderma and gingivitis. Paresthsias and calf tenderness are also not uncommon.

III. *Ameliorative measures.*—Multivitamin tablets have been distributed during diets and nutrition surveys.

IV. *Field observations.*—There is one family where four members got the disease in the same month. *Lathyrus sativus* is grown in the districts of Rewa and Satna along with wheat and Bengal gram. The proportion of *Lathyrus sativus* varies in different fields. In some of the fields it may amount to 50 per cent of the other crops.

Miscellaneous.—Five samples of *Lathyrus* seeds with a note on storage conditions of each sample and thirteen samples of serum for calcium estimation have been sent to Prof. Sadasivan, Director, Botanical Laboratory University of Madras. Three samples of *Lathyrus sativus* have been sent to the Central Food and Technological Institute, Mysore for analysis and some samples to Dr. C.G.S. Iyer, Senior Research Officer, N.P.U., Bombay for animal experimentations.

Materials of one case was sent to Bombay for autopsy. Reports are awaited. It was a case of lathyrism associated with symptoms suggesting pressure on the cord, perhaps by a growth.

203. Investigations on lathyrism under Professor T.S. Sadasivan at the University Botany Laboratory, Madras.

Samples of seeds obtained from Vindhya Pradesh, Madhya Pradesh, Orissa and Assam were screened for internal and external mycoflora. The predominantly occurring fungi include species of *Aspergilli* and *Penicillia*. Single spore cultures of all isolates and identification of the species are in progress.

Serum samples of healthy and diseased persons on identical dietary habits, obtained from Gandhi Memorial Hospital, Rewa, were analyzed for serum calcium by both flame photometry and chemical methods. No conclusive stand could, however, be taken from the data obtained about the significance of calcium status with reference to the disease and analysis of a large number of serum samples is indicated.

Chromatographic analysis of 10 samples of seeds were made for free amino acids in the dry seeds as such. Alanine, aspartic acid, cystine, proline and threonine were present in the majority of the samples tested. Two samples, one from Rewa and the other from Puri, Orissa showed glycine instead of threonine.

Ash analysis by flame photometry of *L. sativus* seeds and other commonly consumed lentils, such as black gram, Bengal gram, green gram and horse gram are in progress.

204. Investigations on lathyrism under Dr. M. Swaminathan at the Central Food Technological Research Institute, Mysore.

1. Chromatographic studies on the non-protein nitrogenous constituents of *Lathyrus sativus* with a view to identify the presence of unknown constituents.

2. Isolation and identification of the toxic principle present in *Lathyrus sativus*.

3. The effect of water soluble extracts of *Lathyrus sativus* on the growth of micro-organisms, with a view to find out whether the toxic factor present in *Lathyrus sativus* has a depressing effect on the growth of micro-organisms.

4. The effect of incorporation of β -amino propionitrile and certain related compounds in the diet of rats on their health and enzymes of liver and nervous tissues.

FLUOROSIS

205. Research on defluorinating substances applicable to waters : development of a defluorinating process for practical application in areas of endemic fluorosis under Dr. S. C. Pillai at the Indian Institute of Science, Bangalore.

The scheme was started on June 29, 1957. Preparation of hydroxy-apatites from phosphatic ores and their possible utilization in removing fluorine from water were considered. Before carrying out trials with naturally occurring phosphatic ores in the country, it was thought desirable to study the efficiency of pure tricalcium phosphate in removing fluorine from tap water treated with known amounts of sodium fluoride. The influence of varying amounts of tricalcium phosphate, the effect of pre-treatment of tricalcium phosphate with alkali, the time of contact with the fluorine-containing water and the influence of varying concentration of fluorine on the efficiency of fluorine removal by tricalcium phosphate were studied. The results indicated (a) that the removal of fluorine by tricalcium phosphate was fairly quick ; (b) that

with increasing amounts of tricalcium phosphate increasing amounts of fluorine were removed; (c) that the fluorine removal by tricalcium phosphate was partly due to adsorption of the entire molecule of sodium fluoride and partly due to exchange of the phosphate in the tricalcium phosphate for the fluorine in solution; (d) that calcium in the tricalcium phosphate could be exchanged for the sodium in the sodium fluoride; and (e) that the efficiency of tricalcium phosphate improved by about 18 per cent on its pre-treatment with sodium hydroxide.

PHYSIOLOGY & PHARMACOLOGY

206. Indigenous drugs enquiry at the School of Tropical Medicine, Calcutta,

I. Continuation of studies on a few fractions of *Rauwolfia serpentina* isolated by counter-current distribution technique :

(a) On the fraction $B_{7,8}$.—This fraction was found to have a cholinergic action, antagonized fully by atropine, on isolated mammalian and amphibian hearts and on isolated guinea-pig ileum. In the latter tissue, it summated with the action of acetylcholine.

Serpine.—This caused a depression of activity of isolated mammalian and amphibian hearts, which was unaffected by atropinization. There was no contraction of isolated guinea-pig ileum even by high doses, and the action of a testing dose of acetylcholine was abolished or reduced in this preparation.

Sarpagine.—In isolated guinea-pig hearts it caused slight depression of amplitude and moderate increase of coronary flow, rate remaining unchanged. Action of acetylcholine was antagonised competitively by sarpagine.

There was no contraction of isolated guinea-pig ileum even after 10^{-4} g./c.c. of sarpagine, actions of histamine and acetylcholine were only slightly antagonised in this tissue.

Thus, it appeared that the fraction $B_{7,8}$ which showed spots in close proximity to those produced by a mixture of serpine and sarpagine on paper chromatographic studies, indicated, pharmacological properties different from those of either serpine or sarpagine on isolated tissues mentioned above.

(b) On the fraction B_1 and ajmaline.—Fraction B_1 , isolated by counter-current distribution technique was shown to be identical with ajmaline by physico-chemical studies. Both fraction B_1 and ajmaline, caused contraction of isolated guinea-pig ileum which was not blocked by atropine or mepyramine. The actions of histamine and acetylcholine were antagonized on this tissue. On washing off and subsequent testing there was a definite potentiation of the action of both histamine and

acetylcholine. The spasmogenic actions of barium chloride and potassium chloride in this tissue were also potentiated by low concentration of both ajmaline and B_1 , but were antagonised by their higher doses. This inhibition of response to barium or potassium chloride caused by higher doses of ajmaline or B_1 took a very much longer time to recover than what was required for the histaminic responses.

Ajmaline also showed some anti-adrenaline action on isolated rabbit auricles, whereas the action of acetylcholine was unaffected in this preparation.

(c) *Rauwolfia* preparations and blood sugar.—Contradictory views appear regarding the action of reserpine i.v. on blood sugar and no report is available regarding the effect of total alkaloids of *Rauwolfia serpentina* on blood sugar. Our observations indicate that in anæsthetised cats; reserpine causes hyperglycæmia, whereas the total alcoholic extract of R.S., causes a steady lowering of blood sugar.

II. Screening of some indigenous drugs on diuresis of rats.

Preliminary experiments on water loaded rats (5 c.c./100 g.) indicate that : (a) Infusion of seeds of *Dolichos biflorus* (orally, 1 g./kg. seeds) caused an increase in the percentage of water excretion, (b) Arborine (from leaves of *Glycosmis pentaphylla* Correa) injected intraperitoneally 20 mg./kg. and (c) watery extract of leaves of *Gymnema sylvestre* (orally 1 g. leaves/kg.) caused some degree of antidiuretic effect. The last observation is interesting in view of the claim of antidiabetic effect of this plant.

III. Pharmacological studies with Aegelin.

This alkaloid was isolated from leaves of *Aegle marmelos* Correa by the Department of Chemistry. The leaves have been attributed reputation in indigenous medicine as a bronchial antispasmodic. Hence, this study was taken up. The alkaloid is soluble with difficulty in various organic solvents which show similar effect as the alkaloid itself in these solvents. Orally and intravenously ægelin does not significantly modify the blood pressure of anæsthetized cats, neither modify the action of adrenaline on blood pressure. Aegelin causes a contraction of denervated nictitating membrane of cats, and an increase of rate and a depression of the amplitude of beats of isolated hearts of rabbit and guinea-pigs, but does not cause significant modification of the action of adrenaline on these tissues. On isolated guinea-pig ileum, the action of histamine and acetylcholine are also not modified. However, dosages of 0.5 to 2 g./kg. of aegelin orally, shows some laxative action in kittens.

207. Indigenous drugs inquiry under Bt.-Col. R. N. Chopra and Dr. I. C. Chopra at the Drug Research Laboratory, Jammu, Tawi.

I. Pharmacological screening of indigenous medicinal plants was continued and following four plants were investigated :

1. *Lochnera rosea* Linn. (Rattan jot).—It is an evergreen shrub which commonly grows in gardens in India. In indigenous medicine,

the plant is used as a remedy for diabetes, uterine disorders and as a tonic and stomachic. An alkaloid resembling serpentine, both chromatographically as well as by similarity of its melting point, has been isolated from the root bark. The pharmacological investigation of the alkaloid showed that it possesses potent hypotensive, sedative and tranquilizing properties as shown by serpentine, one of the alkaloids isolated from *Rauvolfia serpentina*. A new source of alkaloid serpentine has thus been discovered. Although the yield is small at present, only 0.1 per cent, it is possible to increase it by cultivation on scientific lines by use of suitable areas and proper manures.

2. *Ipomea palmata* (Kaladana).—It is a perennial twiner which is cultivated throughout warmer parts of India. The seeds are mostly used as a purgative. Murication A and B and a fixed oil were isolated from the seeds. The extract in a dose 2 mg/kg. given by mouth produced mild purgative action in albino rats. Murication A in a dose of 0.5 mg./kg. showed well marked purgative activity, while the fixed oil from the seeds and Murication B produced no purgative effect.

3. *Bergenia ligulata* (Pakhanveda).—It is a perennial herb which is found in temperate Himalayas. The root is commonly used as laxative, analgesic, diuretic and abortifacient. It is prescribed for the treatment of piles, tumours, urinary troubles, dysentery and menorrhagia. Preliminary pharmacological studies of the alcoholic extract of the roots have shown that it has not a marked action on cardiovascular and respiratory systems. The extract produced definite sedative effect on central nervous system. The intestinal smooth muscle is relaxed.

4. *Verbascum thapsus* (Gidar tamakoo).—It is an erect herb, found in temperate regions of Himalayas. The leaves are hot, anodyne and narcotic. They are useful in chest complaints, gout, diarrhoea, bleeding from lungs and bowels. Seeds are considered aphrodisiac, narcotic and febrifuge. The pharmacological investigations of the alcoholic extract of the whole plant show that it has not a very marked action on the cardiovascular and respiratory systems. The action on the plain muscle of intestine is well marked, as these are relaxed even after induced myogenic and neurogenic spasms. The extract produced sedative effect on central nervous system in small doses.

II. SCREENING OF ANTIBACTERIAL PROPERTIES OF INDIGENOUS DRUGS

The screening of indigenous plants alleged to possess antibacterial properties was continued and alcoholic and ethereal extracts of the different parts of the following plants were tested *in vitro* against the representatives of Gram-negative and Gram-positive organisms and *M. tuberculosis*.

- | | |
|---------------------------|--------------|
| 1. <i>Capsicum nigrum</i> | (Kali mirch) |
| 2. <i>Cocos nucifera</i> | (Nariyal) |
| 3. <i>Punica granatum</i> | (Anar) |

4. <i>Coriander sativum</i>	(Dhania)
5. <i>Ficus bengalensis</i>	(Barh)
6. <i>Ficus glomerata</i>	(Rumlal)
7. <i>Cassia auriculata</i>	(Chevamranga)
8. <i>Plantago major</i>	(Bartange)
9. <i>Peganum hermala</i>	(Harmal)
10. <i>Pinus longifolia</i>	(Chir)

In preliminary studies the extracts of *P. granatum* and *Peganum harmala* have shown definite antimicrobial activity. All other plants showed little or no antibacterial action.

The extracts of *P. granatum* exhibited a specific tuberculostatic action. The ethereal extract of the seeds inhibited the growth of *M. tuberculosis* in a concentration of 80 μ g/c.c. The action of the other parts of the plant also showed good anti-bacterial activity against other test organisms.

208. Inquiry on investigation into the study of the Adrenal cortical hormones under Dr. B. Mukerji at the Central Drug Research Institute, Lucknow.

In the adrenal cortex of immature mice there are two distinct zones upon which luteinizing hormone (LH) and adrenocorticotrophic hormone (ACTH) of the pituitary exert independent trophic influence. In view of this, it was thought worthwhile to test the validity of the theory proposed on the results of the experiments with rats (*vide* I.C.M.R. Annual Report, 1956) by studying the effect of graded doses of testosterone propionate on the adrenal cortex of mice.

The results of the investigations on mice (*vide* I.C.M.R. Annual Report, 1957) validates the theory that androgen influences the adrenocortical activity through multiple pathways. The two principal of these are : (a) Through an augmentation of pituitary adrenocorticotrophic hormone (ACTH), and (b) through a suppressive influence on luteinizing hormone (LH) production by the pituitary. Further, the nature of response of the adrenal cortex to androgens (either stimulatory or depressive) is determined by the dosage and duration of androgen treatment.

The view that testosterone does not stimulate the secretory activity of the adrenal cortex through its anabolic activity, (Roy *et al.*, *Ind. Jour. Med. Res.* 45, 237, 1957) is further established by the use of a primarily protein-anabolic testosterone, 19-nortestosterone. The administration of the latter in graded doses does not cause any change in the adrenal cortex. It is, however, interesting that 19-nortestosterone, like testosterone itself, can antagonise the anti-adrenal effects of cortisone.

- 209.** Study on the anti-veratrinic, anti-accelerator and anti-arrhythmic activity of indigenous and synthetic drugs, and to determine the action of anaesthetics and pre-anaesthetics on cardiac automaticity and conductivity under Dr. R.B. Arora at the All-India Institute of Medical Sciences, New Delhi.

1. To prevent cardiac catastrophies during general anaesthesia the effect of volatile liquid anaesthetics on the automaticity of S-A and A-V node was studied. In all 112 experiments were done on dogs.

The rate of impulse generation in presence of S-A rhythm was accelerated by ether and ethyl chloride. This accelerator response was due to depression of nervous cardiac inhibitory influences, whereas chloroform produced slight initial stimulation followed by strong inhibitory action.

The automaticity of A-V node was found to be more susceptible to the depressant action of chloroform, ether and ethyl chloride. The inhibitory response on A-V nodal rhythm was seen more frequently and was of a greater degree than on S-A nodal rhythm.

Chloroform exerted much more depressant action on cardiac rhythmicity. This inhibitory or depressant action appeared to be due to direct depressant action of chloroform on myocardium as observed in heart lung preparation of dog.

Electrocardiographic changes suggested direct depressant effects of these agents on heart.

Since ether is supposed to be a safe anaesthetic and to produce no deleterious effect on heart in clinically effective dosages, the development of A-V nodal rhythm, as judged by the absence of P wave in the electro-cardiogram encountered during administration of ether, is an important observation. Further work on blood concentration will show the safest dosage range of this anaesthetic.

2. Considering the psychosomatic basis of cardiac arrhythmias, some tranquilizing agents (chlorpromazine, reserpine, rauwiloid, benactyzine and meprobamate) have been investigated in ventricular tachycardia following myocardial infarction by the technique of Harris and Kokernot in 31 dogs. This arrhythmia aetiologically resembles a type of arrhythmia occurring in man following myocardial infarction. In addition, 53 experiments were done on the (a) refractory period of isolated rabbit auricle. (b) aconitine-induced auricular fibrillation, and (c) auricular flutter induced by injury-stimulation method. Chlorpromazine, rauwiloid and benactyzine were found superior to quinidine statistically and deserve clinical trial.

3. 211 experiments on the antiveratrinic action of some anti-histaminic compounds (synopen, mepyramine maleate, promethazine-hydrochloride) some tranquilizing agents (benactyzine, meprobamate, chlorpromazine), and two alkanol esters (McN-120-41, McN-140-11), were done and correlated with their antiarrhythmic action, in an attempt to further establish the antiveratrinic activity as a common property of and a method for screening antiarrhythmic drugs.

4. Heart rate is, however, one of the important mechanisms which adjusts the minute output to the body needs. In heart lung preparations of dogs, anti-accelerator action of chloroquin, a proved antiarrhythmic was investigated, it was found to be of the same type as that of quinidine and quinine. The action is similar to that of veratrimine, in that it sets in abruptly, is persistent and is not prevented or abolished by atropine, but it is less selective.

5. Antiarrhythmic action of certain antihistaminic drugs in isolated rabbit auricle has been reported by Dews and Graham (1946), Dutta (1949), and Mc-Cawley *et al* (1950). Unfortunately, the isolated rabbit auricle is not a very dependable preparation for screening of antiarrhythmic drugs. Hence, the necessity for further investigation. Four antihistaminic drugs, pyribenzamine, synopen, mepyraminic maleate and promethazine hydrochloride were investigated for their antiarrhythmic activity. The techniques employed simulate clinical arrhythmias. Pyribenzamine and synopen are superior to quinidine and deserve clinical trial.

210. Inquiry on the study of anthelmintic action of shell oil of cashew nut under Dr. N. V. Bhaduri at the School of Tropical Medicine, Calcutta.

The anthelmintic properties of the shell oil of cashew nuts were first pointed out by Leao and Eichbaum (1947) when they used it on dogs and found it effective against their hookworms and roundworms. The oil was next used by Eichbaum *et al.* (1950) on a small number of men and its action against the hookworms, roundworms and whipworms was claimed. Bhaduri and Sanyal (1952) took up the study on the anthelmintic action of the shell oil of Indian cashew nuts and their preliminary study showed that the oil was active against roundworms, hookworms and *Fasciolopsis buski*. The oil was irritant to the skin and mucous membranes but was a safe and non-toxic remedy and could be used on cases where few of the standard anthelmintics could be used. Further study by Bhaduri *et al.* (1953, 1954) showed that the shell extract was effective not only against the worms mentioned before but also against *Hymenolepis nana*, *H. diminuta* and some degree of action was noted against *Enterobius vermicularis* and *Strongyloides stercoralis*. Against *Tænia saginata*, *T. solium* and *Trichuris trichiura* the oil appeared to be ineffective. The only drawback was that the extract was a thick viscous liquid and had irritant action on the skin and mucous membranes and had to be put in hard gelatin capsules to be used. This was of much inconvenience for children and also for mass therapy. When the extract was mixed with milk, not much irritation was noticed but much of the anthelmintic action was lost.

Study of the chemistry of the shell oil showed that it was composed of cardol (5-pentadecadienyl resorcinol), anacardic acid (2-hydroxy-6-n-pentadeca-d-enyl benzoic acid) and cardanol (3-pentadecadienyl phenol). When the extract is obtained by roasting the shell much of the anacardic

acid decomposes into cardanol. Separation of the fractions was then undertaken and methods were devised for obtaining cardol and cardanol in pure forms suitable to be used on human beings for the study of their anthelmintic properties. The fraction, cardol, a thick dark brown liquid, irritant to the skin and mucosa like the whole extract, was also found to be a non-toxic, well tolerated remedy active against roundworms, hookworms, *Hymenolepis nana*, *H. diminuta* and *Enterobius vermicularis* (Bhaduri *et al.*, 1954). It could be used on very run down cases and also in pregnancy without any deleterious effects.

The fraction cardanol, is also a thick dark brown viscous liquid irritant to the skin and mucosa like the whole extract or cardol. This fraction also has been found to have anthelmintic properties like the whole extract or cardol but was distinctly weaker (Bhaduri *et al.*, 1956). Thus, the whole extract, cardol or cardanol, all were good anthelmintics with a broad range of action and were safe and well-tolerated substances. Their liquid form was the only drawback for a large-scale use. Attempts were next made to obtain some solid derivatives from them and cardol was first taken up. It was hydrogenated in the laboratory under normal temperature and pressure and a solid power was obtained. This is being tested for its anthelmintic action it has been found to be a safe and non-toxic substance with action against hookworms and roundworms. The study is in progress.

Hydrogenation of cardanol and anacardic acid has also been done and powdery substances obtained. Salts of anacardic acid and their acetyl derivatives have also been prepared for study, as Eichbaum (1947), Findlay (1950) and Peach and Tracy (1956) have all hinted at their anthelmintic properties. Some of these have already been used on a few human cases and have been found to be well-tolerated without production of any toxic symptoms.

211. Inquiry into the influence of anti-rheumatic substances on the hypothyse o-adrenocortical axis under Dr. G.K. Karandikar at the Medical College, Baroda.

Work with sodium salicylate, cincophen, aminopyrine, butazolidine and colchicine using adrenal cholesterol and ascorbic-acid contents, total eosinophil count and histological picture of lymphoid tissue as indices of adrenocortical activity has been done.

Sodium salicylate 0.3 g./kg. i.p. depleted only the ascorbic-acid content of the adrenal gland at both the 2nd hour and 6 hour intervals. Butazolidine 50 mg./kg. depleted only the cholesterol content of the adrenal glands at the 2nd hour and produced only eosinopenia at the 4th and 6th hours. Colchicine 1 mg./kg. depleted only the cholesterol content of the adrenal glands at the 4th and 6th hours. Aminopyrine 150 mg./kg. depleted the cholesterol content of the adrenal glands and produced eosinopenia at the 2nd hour.

212. Inquiry on anticonvulsants and antifibrillatory action of drugs in relation to acetylcholine synthesis under Dr. B.C. Bose at the M.G.M. Medical College, Indore.

The object of the work was to study the mechanism of anti-convulsant and anti-fibrillatory actions of drugs in arresting the cerebral and cardiac arrhythmias, respectively. As these arrhythmias have been shown to be influenced by the rate of synthesis of acetylcholine in the brain and heart tissues, it was proposed to investigate if the group of drugs arresting these irregularities of rhythm would also affect the synthesis of acetylcholine in these tissues.

In the last year's report on effect of anti-convulsants drugs fed to rats, a reduction in the acetylcholine content was observed. During the year under review a series of detailed acute experiments was carried out under local anæsthesia—chipping out a square piece of parietal and temporal bones and after removal of a small portion of cortex the cavity being packed by vaseline gauze and wound sutured. Drug injected and after two hours, intact side operated for second ablation. Results of acetylcholine content in both control drug and treated rats are summarised in Tables I and II.

TABLE I

Showing the average acetylcholine content in cerebral cortex removed at varying intervals.

(The figures are the averages of 8 experiments in each case.)

	Initial	After 1 hr.	After 2 hrs.	After 3 hrs.	After 4 hrs,
Ach content in μg./g.	3·18	3·04	2·74	2·60	2·42
Standard error.	0·26	0·14	0·09	0·10	0·17
Percentage reduction	—	4·50	12·50	18·50	24·50

TABLE II.

Showing the average acetylcholine content of brain tissue of rats after graded doses of hydantoin derivatives.

Drug	Dose	Mean Ach control μ g./g., C_1	Postulated mean Ach after 2 hrs., μ g./g., C_2	Mean Ach after drug μ g./g., 5	Difference 4 & 5 6	$\sqrt[3]{SE^2(C_2) + SE(t)}$ 7
I	2	3	4	5	6	7
Dilantin sodium	4	2.76 $\pm$.22	2.41 $\pm$.17	1.98 $\pm$.11	0.43	0.40
	6	3.16 $\pm$.24	2.77 $\pm$.21	2.00 $\pm$.21	0.77	0.56
	8	2.80 $\pm$.17	2.45 $\pm$.14	1.47 $\pm$.17	0.98	0.40
Thiantoine sodium	4	.280 $\pm$.17	2.45 $\pm$.14	2.09 $\pm$.11	0.36	0.34
	6	.301 $\pm$.30	2.64 $\pm$.26	1.94 $\pm$.20	0.70	0.62
	8	.289 $\pm$.17	2.53 $\pm$.24	1.81 $\pm$.22	0.72	0.62
Mesantoin	4	.300 $\pm$.24	2.63 $\pm$.20	2.04 $\pm$.19	0.57	0.52
	6	.295 $\pm$.26	2.59 $\pm$.22	1.82 $\pm$.24	0.77	0.62
	8	.328 $\pm$.28	2.87 $\pm$.24	1.64 $\pm$.14	1.23	0.52

On completion of the investigation with hydantoin derivatives the study was extended to paludrine and Chlorpromazine, the former belonging to the group of antifibrillatory and the later to the antihistaminic drugs. The findings are represented in Table III and IV.

TABLE III.

Showing effect of paludrine on the acetylcholine content in brain tissue in rat.

Dose	Control Ach content μ g./gm.	After paludrine	Percentage reduction	Number of Experiments.
1 mg./kg.	2.27	1.69	25.4 %	6
1.5 mg./kg.	2.50	1.75	30.0 %	6
2.0 mg./kg.	2.72	1.69	38.6 %	8

TABLE IV.

Showing the effect of chlorpromazine on the acetylcholine content in brain tissue in rats.

Rat number	Dose	Ach in μ g./g. Control	Ach in μ g./g. after chlorpromazine.	Percentage reduction
1	1 mg./kg.	3.90	2.80	42.9
2	"	3.56	2.50	35.9
3	"	3.40	2.30	37.4
4	"	3.22	1.70	48.9

It will be observed from the above tables that both paludrine and chlorpromazine produce a significant reduction in acetylcholine content in brain tissues.

213. Inquiry on canine and human gastric function in summer and winter under Dr. B.S. Kahali at the S.M.S. Medical College, Jaipur.

Human gastric function.—Ten healthy volunteers of age group 18 to 28 without any detectable abnormality of any function were selected for investigation of gastric function. The investigation on these ten

subjects during rains, winter and summer has been completed. Additional 20 subjects have been selected for study during the current year and the gastric secretory response of these subjects during the last rains has been completed.

Method used.—The secretory function of the stomach was estimated by the method of fractional gastric analysis of Rehfuß.

Choice of meals.—Two non-protein test meals—7 per cent alcohol meal and 2 per cent pectin meal sweetened with sucrose were tried. The alcohol meal is the better of the two on account of ease of preparation and administration. It is also harmless: pectin meal in some subjects causes griping and diarrhoea.

Variability of the gastric secretory response from day to day in normal persons.—Considerable, day to day, variation of the gastric secretory response to alcohol test meal and also to histamine injection in the same person has been observed. A single fractional gastric analysis is of no value in estimating the functional ability of the stomach.

In this work average of the results of three fractional gastric analysis repeated on three consecutive or alternate days has been taken as a measure of gastric-response.

Estimation of pepsin.—Pepsin has been estimated by a modification of Hunt's method of using human plasma or serum protein as a substrate. The method is costly but easier to perform and gives consistent results. Egg white and bovine serum albumin are useless as suitable protein substrate. Pepsin content has no correlation with the free acid concentration, it remains at a higher level in the later samples of gastric contents when usually free acid concentration tends to decline.

Pepsin content has been estimated in the pooled sample of gastric contents.

Seasonal variation.—Secretory response of the stomach is the highest in winter, lowest in summer and intermediate in rains.

Motor functions studied radiographically after barium meal.—In general, the motor function of the stomach in these subjects is more intense in winter than in summer. The increased motor activity in winter is much less marked than the increase in the secretory response of the stomach in winter.

Canine gastric function.—Preparation of fundic pouch in dogs by the original technique of Pavlov may not always give an innervated pouch. Thomas's modification of the technique as further improved by Wakman and Nasset usually always gives an innervated pouch.

It is very difficult to keep these animals with cannulated fundic pouch in good health for a long time for prolonged observation. Infection of the cannula track was always present. Maintenance of proper nutritive condition was not possible for more than 3 to 4 weeks. By eight weeks they became unfit for experimentation.

Improved operative technique and post operative treatment, nursing and dieting is being tried to increase the survival period of the operated dogs. An alternative method of studying the secretory response of the whole stomach by suitable gastrostomy is also being tried.

214. Neuro-physiology Research unit under Dr. B.K. Anand at the All-India Institute of Medical Sciences, New Delhi.

The studies of the previous years on the hypothalamus as well as the limbic system of the brain have been further continued. The following conclusions have been drawn as a result of these further studies.

(1) NERVOUS REGULATION OF FOOD INTAKE :

(i) *Ablation studies* :—A total of 41 monkeys and 13 cats have been observed following bilateral surgical as well as electrolytic lesions produced in different regions of the frontal or temporal lobes. As a result of these studies, it has been conclusively shown that frontal lobe lesions produce a decrease in food intake if the orbital surface is involved in the lesion. If the lesion is restricted only to the anterior portion of the frontal lobe, there is a definite increase in food intake. Temporal lobe lesions, on the other hand, produce an increase in food intake provided the amygdaloid region is not involved. If amygdaloid region is involved, then there is a complete temporary aphagia lasting only for the first few days after which there is hypophagia, which ultimately may change to hyperphagia if the lesion is extensive and involves the other temporal lobe structures too.

(ii) *Recordings from the feeding centres* :— In order to find out the mechanism through which the two hypothalamic centres ('feeding' and 'satiety') are activated, electro-encephalographic studies have been carried out from electrodes implanted in these centres. After recording their normal potential activity, changes have been produced in blood chemistry to see if such changes in any way affect the activity of these centres. Although definite changes in the activity of these centres have been observed in very few animals, the results obtained as yet have not reached the conclusive stage.

(2) RESULTS OF RESTRICTED SURGICAL LESIONS INVOLVING PORTIONS OF THE LIMBIC SYSTEM OF THE BRAIN :

Thirty-one monkeys and 18 cats have been used, out of which 22 monkeys and 10 cats survived the operation. Seven monkeys and 4 cats had lesions in frontal lobe and 15 monkeys and 6 cats had lesions in temporal lobes. In all these animals, the lesions were restricted either to the frontal tips, orbital surface of the frontal lobes, anterior cingulate gyri, temporal tips, amygdaloid nuclei, hippocampal formation, or to the uncus. Few had restricted lesions only on the lateral surface of the temporal lobes.

The responses observed are summarised below :—

1. Fearlessness was observed after lesions involving the amygdaloid and hippocampus regions of the temporal lobes and the frontal tips. Confusion and shyness of test objects was shown to be due to involvement of orbital surface of frontal lobes and head of caudate nuclei.
2. Non-aggressiveness and docility resulted from lesions to the amygdaloid and hippocampus regions. Lesions of the frontal tips produced animals friendly towards known individuals and aggressive towards strangers. Lesions in the anterior cingulate gyri did not alter the range threshold significantly.
3. Lesions of the orbital surface, which also involved the heads of caudate nuclei, produced an aimless persistent hyperactivity.
4. Some animals after temporal as well as frontal lesions developed 'oral tendencies'. They would smell everything presented to them and put it into their mouth.
5. Some monkeys having lesions in the temporal tips and amygdaloid hippocampus regions developed marked hypersexuality.
6. Blood pressure tended to drop and rectal temperature registered a small fall after lesions of the frontal tips and orbital surface. Lesions of amygdaloid and hippocampus regions produced an opposite effect. Changes in the heart rate were quite variable.
7. Respiration rate tended to drop after both frontal and temporal lesions.
8. Hyperaemic and ulcerative changes in the gastro-intestinal mucous membrane were seen post-mortem after most of these lesions. Ballooning of the gut was observed both after lesions in the amygdaloid hippocampus regions and the frontal tips.
9. After both frontal and temporal lesions changes in the blood levels of sugar and sodium were produced; majority showing a rise and some a fall. In addition to autonomic system, endocrinal involvement is suggested.
10. After many such lesions there was slight decrease in serum proteins with a slight rise in globulins, disturbing the albumen: globulin ratio. Involvement of liver is suggested.

(3) HYPOTHALAMIC REGULATION OF WATER INTAKE.

Studies on water and food intake have been carried out in response to bilateral hypothalamic lesions. It has been observed that lesions in the 'feeding centre', in addition to complete aphagia, also produced

complete adipsia. Lesions in its adjacent regions produced hypodipsia irrespective of increase in food intake. On the other hand, lesions further removed from this region do not significantly change the water intake, inspite of changes in food intake. After the hypothalamic lesions the animals also failed to show any correlation of water with the food intake, which is generally observed in normal animals. It has therefore been concluded that a close relationship exists between water regulation and energy metabolism, and it seems possible that an integrated regulation of both these essential functions is situated in the same area within the hypothalamus.

(4) ACETYLCHOLINE AND GLUTATHIONE ESTIMATIONS IN HYPOTHERMIA.

This study has been further continued and it has been observed that in dogs, in which hypothermia is produced, the acetylcholine as well as glutathione register a definite and significant fall in the cardiac tissue, as well as in the hypothalamus and frontal lobes of the brain. On the other hand, as a result of hypothermia both the acetylcholine as well as glutathione contents of the temporal lobe significantly increase. As a result of these studies it has been concluded that the ill effects of hypothermia, such as the production of ventricular fibrillation as well as the production of irreversible hypothermia, may have something to do with the fall of acetylcholine and glutathione to levels below a certain optimum. This view is supported by the fact that both these substances are essential in the proper functioning of the cells in conditions of relative anoxæmia. Also, the observations that these substances are increased in the temporal lobe during hypothermia, suggest that the limbic structures in this lobe possibly are involved in the regulation of body temperature and therefore this may be a compensatory response.

(5) PHYSIOLOGICAL CONNECTIONS BETWEEN THE CEREBELLUM AND THE LIMBIC SYSTEM OF CEREBERUM.

As a result of studies carried out by many previous workers, it had been established that, although the neocerebellar regions have got both to and fro connections with the cerebral cortical structures, the paleocerebellar structures project only into the reticular formation of the brainstem. No reports have appeared so far showing any physiological projections from the paleocerebellum into any cerebral regions.

The evoked potential studies have been carried out in dogs in whom, under the effect of intraperitoneal Dial anæsthesia, different regions of the cerebellum have been stimulated by silver wire electrodes and action potentials recorded from different parts of the Limbic System of the cerebrum by means of a cathode-ray oscilloscope. It has been observed that the paleocerebellum (old parts of cerebellum) has got extensive connections with the different limbic lobe structures of the frontal and temporal lobes.

Previous studies had established paleocerebellar connections only with the reticular formation of the brain-stem (activating region of magoun-centrencephalic centre of Penfield). These regions of the brain-stem have been shown to activate the cerebral cortex in the

awakening response as well as influence the postural activity of the body. Similar functions were, therefore, attributed to paleocerebellum. Recent studies have shown interconnections between the activating region and the limbic system, and the limbic system has, therefore, been shown also to be involved in the conscious response of the central nervous system. The present study which establishes the paleocerebellar connections with the limbic system (probably through the activating system) is, therefore, of much added interest.

(6) NERVOUS CONTROL OVER GASTRIC SECRETION.

The work was further extended to see the effects of stimulation of the different limbic structures of the cerebrum on the gastric secretory activity, studied by making gastric pouches. It was observed that stimulation of the amygdaloid nuclei, the tip of the temporal lobe, and the posterior orbital surface of the frontal lobe, produced a marked increase in the volume of gastric secretion, in its acidity (both free and total), and its pepsin contents. Also, this led to the appearance of occult blood in gastric juice, alongwith appearance of erosions and hyperæmic patches in the gastric mucosa. Stimulation of anterior cingulate gyrus, or the hippocampus, on the other hand, did not produce any marked changes. Thus, the stimulation of the structures listed above produces the same type of response as the stimulation of the anteromedial regions of the hypothalamus and the tegmentum. It has, therefore, been argued that the activation of these limbic structures produce their effects through the hypothalamic regions.

(7) STUDIES ON PHYSIOLOGICAL CHANGES IN YOGIS.

Drs. Wenger and Bagchi from U.S.A. visited this country during this year under the joint auspices of the Indian Council of Medical Research and the Rockefeller Foundation. During their stay in Delhi during the months of February and March, this Unit collaborated with them in their studies. After they left this country, some more subjects have been investigated.

(8) This Unit has also collaborated in certain investigations beings conducted at the Malaria Institute of India, where the effects of heavy malarial infections on liver damage are being studied. In some of these monkeys, lesions have been produced in the hypothalamic regions to study if this autonomic region of the brain is in any way involved in the liver damage,

215. Studies on the growth of resistance in micro-organisms against antibiotics under Dr. S. K. Bose at the University College of Science and Technology, Calcutta.

(1) The distribution of resistance in a population of normal cells towards ninhydrin follows the same general pattern as has been observed in connection with penicillin and formaldehyde. In other words, a

normal sensitive population may be characterized as having majority of cells possessing average resistance and the minority increasingly high resistance.

(2) The distribution of resistance in a population of resistant cells is altered in the sense that all the individuals in a population exhibit a steadily increasing degree of resistance, and therefore, resemble the minority of a normal sensitive population in this respect. This has been found to be the case with penicillin, formaldehyde and ninhydrin.

(3) Resistant isolates at a concentration as high as 8,000 units per ml. of penicillin has been obtained.

(4) The frequency of occurrence of higher resistant types decreases both in sensitive and resistant populations and the isolation of a given resistant type, therefore, depends on the size of the implant inoculum.

(5) It appears that not only penicillin can induce penicillin resistance but substances other than penicillin can do so provided the degree of resistance attained is of a lower order.

(6) Progressive increase in penicillin resistance is not accompanied by simultaneous increase in resistance against substances other than penicillin which shows that it is not a case of cross-resistance.

216. Inquiry on the role of electrolyte imbalance in hypertension under Dr. Inderjit Singh at the S. N. Medical College, Agra.

The effect of adenosinetriphosphate on active relaxation of smooth muscle has been tested. In 12 experiments, ATP produced active relaxation of frog's stomach muscle to the same extent as adrenaline of living muscles. But in subsequent experiments, numbering over 60, ATP produced no relaxation at all. The cause of this is being investigated.

217. Inquiry on screening of penicillin compounds under Dr. P. D. Kulkarni and Dr. K. Ganapathi at the Research Laboratories of the Hindustan Antibiotics, Pimpri.

Sixteen compounds of penicillin were undertaken for investigation. They belong to three different series as mentioned below :

- (a) N-N dialkyl ethylene diamines by introducing groups of homologous series, from C_2 to C_{12} .
- (b) N-N diaryl diamines by substitution in benzene nucleus.
- (c) Steroid compounds.

These compounds were investigated for :

(i) *Potency* : (a) By microbiological assay—*B. subtilis* being used as test organism.

(b) Iodometrically.

(ii) *Stability* (In artificial gastric juice pH 4.5) : Suspensions of the salt were prepared in buffer pH 7 (control) and artificial gastric juice. Both were incubated at 37°C for 2 hours and bioassay was done.

(iii) *Toxicity*.—Tested in mice weighing 25 g. by intraperitoneal introjection of 1,000 units suspended in 1 c.c. normal saline. Tween 80, 0.1 c.c. was used as suspending agent.

(iv) *Blood serum concentrations*.—Suspensions containing 100,000 units, prepared in 2 c.c. of phosphate buffer (pH 7) and 0.1 c.c. Tween 80, were injected intramuscularly into rabbits weighing 2 kg. to 3 kg. Two or more rabbits were used for each salt.

Rabbits were bled at different intervals. (24—48—72 hours) and the serum concentrations of penicillin were estimated, till the blood-serum level fell below 0.01 units per c.c.

Method used for the microbiological assays of serum levels.—Large 10"×10" glass plates pressed in metal rims were cleaned with alcohol, flamed and levelled on a levelling table. 125 c.c. of melted 'seed layer agar' inoculated with 10 c.c. of overnight broth-culture of *S. lutea* (test organism), was poured into each plate. Plates were allowed to set and covered with lids. Sterile heatly cups were placed on the agar in 5 rows of 5 cups each. Cups were filled with penicillin G. standards of different unitage (from 0.02 units/c.c. to 0.5 units/c.c.) and rabbit sera. The plates were then put in cold room for 2 hours to allow diffusion and incubated at 37°C. for 18 hours. Zones of inhibition were read and from the 'standard curve' concentrations of unknown sera were found.

Results : (1) N-N' Di-N-octyl E.D. and (2) N-Benzyl Cholesterylamine show an effective blood level for over 5 to 6 days. They are therefore being studied in greater detail.

218. Inquiry to study the diuretic and anti-diuretic Ayurvedic remedies under Drs. G. K. Karandikar and O. D. Gulati at the Medical College, Baroda.

Following norms have been established in conscious male rats (140 g. to 240 g.) in metabolism cages and anæsthetized female dogs (weight 8 kg. to 15 kg. and anæsthesia Pento barbitone sodium 30

mg./kg.). The animals were hydrated by the oral administration of 50 c.c. kg. of tap-water prior to the start of the experiment.

(a) Total urinary excretion :

(i) Rats 34·3 c.c./kg.

(ii) Dogs 26·3 c.c./kg.

(b) Maximum excretion rate :

(i) Rats 73 minutes.

(ii) Dogs 132 minutes.

(c) Urinary chloride content :

(i) Rats 0·1 per cent.

(ii) Dogs not yet established.

Some diuretic studies on *Punarnawa* extract have been made in comparison with mersyl. Extracts of *Punarnawa* and *Gokhru* are ready and arrangements are being made to start the work on students also.

219. Inquiry on transmethylation of nor-adrenaline to adrenaline in relation to cardiac arrhythmias under Dr. B.C. Bose at the M.G.M. Medical College, Indore.

The object of the work was to study the process of transmethylation of nor adrenaline to adrenaline and vice versa and to find out the effect of the anti-fibrillatory drugs on this transmethylation process.

A series of *in-vitro* investigations was carried out on the adrenal glands, heart, liver tissues of freshly-killed rats, rabbits and dogs. The different tissues were incubated with methyl donors, such as methione, choline, vitamin B₁₂ for four hours at 37°C., after which the proportions of adrenaline and nor-adrenaline were estimated by the following methods :-

(a) Rat-uterus method for adrenaline assay.

(b) Rat ascending colon method for nor-adrenaline assay.

The results of transmethylation experiments are summarised in Table I:—

TABLE I.

Showing the percentage methylation of nor-adrenaline to adrenaline in heart, liver and adrenal tissues.

Tissue used	Methylating agents used.	Number of experiments done.	Positive methylation	Percentage methylation observed
Heart	Choline	15	5	33%
"	B ₁₂	15	5	33%
"	A.T.P.	15	5	33%
"	Methionine	10	4	40%
Adrenal glands	Choline	10	4	40%
"	B ₁₂	10	7	70%
"	A.T.P.	8	5	65%
"	Methionine	10	5	50%
Liver	Choline	5	2	40%
"	B ₁₂	5	1	20%
"	A.T.P.	5	1	20%
"	Methionine	5	2	40%

From Table I it will be observed that all the four methylating agents caused transmethylation of nor adrenaline to adrenaline in all the three tissues under investigation, maximum amount of transmethylation occurring in adrenal glands.

Side by side with the above work on heart, liver and adrenal tissues, it was considered advisable to study the transmethylation process occurring in at ileum and colon, in which organ it is known that the inhibitory ratios of adrenaline and nor-adrenaline may vary within wide range.

It was, therefore, considered necessary to correlate these variable responses with the active transmethylation process occurring in these tissues during contractions.

TABLE II.

Showing the inhibitory ratios of adrenaline and nor-adrenaline in relation to acetylcholine content of rat duodenum, jejunum and colon.

Number of observations	DUODENUM		JEJUNUM		COLON	
	Ach μ g/g.	Ratio of Adrenaline Nor-adrenaline	Ach μ g. g.	Ratio of Adrenaline Nor-adrenaline	Ach μ g/g.	Ratio of Adrenaline Nor-adrenaline
1	1.25	0.50	2.50	0.81	1.25	0.31
2	2.00	0.76	3.50	1.30	1.40	0.66
3	5.00	1.20	4.53	1.50	1.50	0.50
4	5.20	1.20	5.00	1.80	1.50	0.44
5	5.42	1.30	5.12	2.10	2.00	0.74
6	6.02	1.20	5.40	1.80	2.50	0.47
7	6.30	1.30	6.51	2.50	5.00	0.60
8	7.50	1.50	8.02	2.80	5.00	0.73
9	9.20	1.50	9.50	2.90	7.50	1.00
10	9.50	1.80	10.50	3.80	9.50	1.80

From the data presented in Table II it will be observed that the average inhibitory ratios for jejunum, duodenum and colon are in correspondence with the acetylcholine concentration in these segments.

It is likely that the greater amount of acetylcholine might probably sensitize the tissues to the action of adrenaline by increasing the transmethylation occurring at the neuromuscular junction of the smooth muscles.

220. Inquiry on "The effect of rutin and related glycosides on the carbohydrate metabolism of liver" under Dr. Mohan Singh Sethi at the Medical College, Amritsar.

Warburg tissue respiration apparatus has been set up and some preliminary experiments have been conducted with rat and rabbit liver slices. Slices from livers of normal animals have been incubated in Krebs' solution and the oxygen uptake compared with that of the slices in Krebs' solution containing hesperidine methyl chalcone. It seems that hesperidine methyl chalcone depresses oxygen uptake of liver slices.

Some experiments have been performed to determine effect of hesperidin methyl chalcone, rutin and quercetin on glycogenolysis. They seem to depress the glycogen synthesis by rat diaphragm slices. Preliminary experiments have not given any reliable conclusions about the effect on liver slices.

221. Inquiry on urinary estimation of catechol amines in normal and hypertensive individuals under Dr. R.S. Grewal at the Medical College, Nagpur.

To start with, the method of Burn (*Brit. Med. Jour.* p. 697, 1953) a slight modification of the original method of Euler and Hellner (*Acta Phys. Scand.*, 22 p. 161, 1951) was employed. Being very elaborate, this method was replaced by that of Euler and Orwen (*Acta Phys. Scand.*, 33, Suppl. 118, p. 1, 1955) and only the results obtained by the latter method have been included here.

MATERIAL AND METHOD

From April 1957 to September 1957 urinary catechol amines have been determined in 25 normotensive individuals. Normotensive individuals diastolic blood pressure below 85 belonged to any one of the following groups. Young medical graduates, medical students, convalescent patients or pregnant women. Normal pregnant women have been taken for study because hypertension due to toxæmias of pregnancy will be studied, and it would be more correct if the urinary catechol amines of toxæmic women are compared with normal pregnant women.

1. *Collection of urine.*—24 hour urine specimen was collected in bottles containing concentrated hydrochloric acid, about 5 c.c. of which is sufficient for keeping 800 c.c. of urine at about pH 3. The acid acts as a preservative for the catechol amines. The urine is stored in a refrigerator when more than 24 to 48 hours elapsed between the collection of the urine and the preparation of the extract.

2. *Preparation of the urine extract.*—The method of Euler and Orwen (*loc. cit.*) has been followed. The 24 hour sample of urine is measured, and out of the pooled sample 200 c.c. urine is taken, the pH is adjusted between 1 and 2 and boiled under a reflux for 20 minutes, the aim being to hydrolyse the conjugated amines into free amines. It is then cooled. Five g. of alumina (for chromatographic use) is added and sodium-hydroxide solution (0.5 N or 2 per cent is added, drop by drop, till the urine gives a greenish colour with B.D.H. universal indicator (approx. pH 7.5). This can be conveniently seen on porcelain tile by mixing 1 drop of indicator solution with 1 drop of urine. The final adjustment to the pH 8.5 is done by using B.D.H. short range pH paper (7.0 to 8.5) and a violet colour develops. It is usually necessary to add a few more c.c. of NaOH solution to keep the mixture at pH 8.5. The urine is filtered in a Buchner funnel using 2 filter papers (Whatman No. 4). The filtrate is discarded. Alumina is washed twice with 10 c.c. to 20 c.c. of distilled water and filtered dry. The catechol amines are then eluted using 0.25 N sulphuric acid, once with 5 c.c. and then with 4.5 c.c. The eluate always had a pH below 4. This is stored in a refrigerator till the bioassay is done (usually period never exceeding one week). Before bioassay the extract is neutralized with 20 per cent analar sodium-bicarbonate to pH 4.0 using bromphenol blue as an indicator. This neutralization is important because highly acidic solutions give rise to depressor responses.

3. *Biological assay on cat blood pressure. (Preparation of the cat).—*Cats of average size (about 2 kg.) have been used. Ether anæsthesia is given to start with and maintained till the animal is made spinal. The blood pressure is recorded from one of the carotid arteries and the femoral vein is employed for making the injections.

It has been observed that the sensitivity of the animal increase to about 30 to 60 minutes after the completion of the preparation. Hence, the assay is usually started after the lapse of the said period.

Assay.—The pressor activity of the urine extract is compared with a standard non-adrenaline bitartrate solution (1 or 2 microgram/c.c. strength is used).

Observations and results : Recovery of added noradrenaline.—To test the efficacy of the extraction process specimen of urine were divided into two parts. To one part are added 100 microgram of noradrenaline. Both parts are then extracted and the difference between the two assays give the amount of noradrenaline recovered. In 10 observations made so far, the recovery values varied from 60 to 70 per cent with a mean value of 65 per cent.

The results of 25 estimations done in normotensive individuals are the lowest daily excretion 27 microgram. Highest daily excretion was 123.1 microgram, mean excretion 68.7 microgram, standard deviation 24.5, standard error of the mean 4.9. The pressor activity is expressed in terms of noradrenaline bitartrate.

222. Inquiry on the effect of œstrogens on histaminolytic activity of plasma and on gastric secretion, under Dr. B.B. Tripathy at the Medical College, Cuttack.

Object of work.—Histaminolytic activity of blood increases greatly in women during pregnancy. During such periods clinical relief is obtained from many disorders, directly or indirectly connected with the phenomenon of allergy and histamine liberation.

Peptic ulcer is one among these, from which remarkable relief of symptoms and freedom from complications is observed during periods of gastation. Relief is also obtained from the above condition when œstrogen therapy is resorted to.

It has been observed that the titre of histaminolytic activity does not keep pace with the level of gonadotrophins in blood or urine. It follows more closely the rise and fall of œstrogenic activity of blood. It is, therefore, proposed here to study directly the effect of œstrogen administration on histaminolytic activity of blood.

Some work has been done on the effect of œstrogens on gastric secretion, mainly because gastric acidity is lower in women in general and during pregnancy in particular. Results have not been equivocal in this respect. In the present work this problem will be studied both in men and animals.

Procedure :

(i) Histaminase content of blood before and after oestrogen therapy will be estimated mostly by the biological method. Some samples will be examined by the volumetric (chemical) method evolved by Kapeller Adler (*Biochem. Jour.*, **48**, p. 99, 1951).

(ii) Gastric secretion will also be studied before and after oestrogen therapy both in men and animals.

223. Inquiry on histamine content and histaminolytic properties of cerebrospinal fluid of animals and humans in health and disease under Dr. P.K. Kar at the S.C.B. Medical College, Cuttack.

Object of the scheme.—It was proposed to determine the histamine content and the histaminolytic property of C.S.F. in animals and in man in health and disease and determine along with the main work, the existence, if any, of a 'blood brain barrier' to histamine, and to study the pharmacological effects of histamine on the central nervous systems.

Pooled specimens of cerebrospinal fluid of normal subjects were incubated for varying periods with a standard dilute solution of histamine acid phosphate. The specimens thus obtained were compared with the standard solution on a piece of guinea-pig's intestine for reduced spasmogenic action as the index of histaminolytic property of the cerebrospinal fluid. Though the tests so far performed cannot be claimed to be accurately quantitative, there are evidences of a gradual but steady histaminolytic action for a period of nearly 3 hours, culminating after that period, in a sharp and steeper action. Quantitative testing and other investigations are in progress.

224. Comprehensive study of the respiratory responses and energy expenditure of expert mountaineers and of other subjects during climbing hills with loads under Dr. H. Saha at the Nilratan Sircar Medical College, Calcutta.

The respiratory metabolism and efficiency of hill-climbing of Sri Tensing Norgay (subject 'D') were compared with 3 other subjects viz.—

Subject 'A'—from Calcutta. Sedentary habit.

Subject 'B'—from Calcutta. Athlete.

Subject 'C'—Native of Darjeeling. Sedentary habit.

The experiments were conducted at Darjeeling (Bar. Pr. 593 mm. Hg; Ambient Temp. 15°C). Each subject carried two different loads (7.3 kg. and 12.8 kg.) and climbed on two different gradients (1 in 8, and 1 in 6) at a constant speed of 2 m.p.h. for 8 minutes. The expired air was collected in a special type of gasmeter (Kofranyi-Michaelis calorimeter) in the steady-state and analysed in Scholander's micro-gas analyser

(Scholander, 1942). The experiments, 16 in all, were arranged in the form of a 4×4 Latin square to facilitate statistical computations.

The results indicate : (1) Sri Tensing Norgay had higher mechanical efficiency compared to other subjects, a difference which became more evident during climbing on steeper gradients with heavier loads. Statistical analysis of the data indicated a significant difference in mechanical efficiency, between 'D' and 'A', a highly significant difference between 'D' and 'C' and a less significant difference between 'D' and 'B'. Subject 'B', is a reputed swimmer and his better efficiency during hill-climbing perhaps could be attributed to his athletic habits. Contrary to expectation, subject 'C' (native of Darjeeling) had very poor mechanical efficiency during climbing.

Ventilatory equivalent of oxygen (volume of pulmonary ventilation required to extract 100 c.c. of oxygen from inspired air) is taken as an indication of respiratory efficiency. Subject 'D' had the highest respiratory efficiency which remained constant in all the experiments. In subject 'C' respiratory efficiency was low at first but improved gradually till with the most strenuous climb his respiratory efficiency was almost as high as that of subject 'D'. The respiratory efficiency of subjects 'A' and 'B' deteriorated considerably with gradually increasing stress of work.

The R.Q. of subject 'D' increased from 0.81 to 0.88, and that of 'C' from 0.89 to 0.96 with increasing severity of work. In subjects 'A' and 'B' the R.Q. approached unity during the most strenuous climb. This means that subjects 'A' and 'B' were much more exhausted while climbing on a steeper gradient with heavier load as compared to subjects 'C' and 'D'. The 'resting' ventilation and oxygen consumption of 'C' and 'D' were higher as compared to those of 'A' and 'B'. One subject from Calcutta (A) showed an increase of 15 per cent in the 'resting' metabolism on the day he arrived at Darjeeling. No generalization is permissible in view of the very limited number of observations.

225. Studies in vascular reflexes in spinal and non-spinal preparations clinical and experimental under Dr. S.R. Mukherjee at the Medical College, Calcutta.

Experimental.—In 20 individual experiments (cats) the following results have been obtained. All the experiments were done under urethane anaesthesia.

- (1) Distention of small segments of ileum isolated from the rest with normal saline at a pressure varying from 40 to 60 mm. Hg. does not cause appreciable rise of blood pressure (more than 10 to 12 per cent) when the buffer nerves were intact; following vagotomy and the occlusion of the carotid arteries, such distension causes marked increase of blood pressure (up to 30 per cent from the basal level).

- (2) Distension of the loop of the jejunum produces changes as described before.
- (3) Distension of the duodenum causes initially marked vomiting and produces reduction in blood pressure when the buffer nerves were intact. Then it is followed by slight rise of blood pressure. Following vagotomy and occlusion of carotids the vomiting usually does not, however, occur and there is no initial depression of blood pressure.

Clinical.—In 20 cases of hypertension, both benign and malignant group, distension of urinary bladder varying from 8 to 16 ounces increases the blood pressure by 15 to 30 per cent.

The mechanism of such rise in blood pressure is now being investigated. The effects of different anaesthesia in influencing these reflexes are also being studied.

226. Inquiry on participation of parathyroid glands in acute systemic stress under Dr. T. H. Rindani at the Topiwala National Medical College, Bombay.

The role of parathyroids in the complex metabolic response to acute non-specific stress has not attracted much attention so far. Since well-marked alterations in calcium and phosphorus metabolism accompany several stressful situations, it was considered pertinent to enquire if and at what stage these glands participated in the calcium phosphorus homeostasis following stress.

The studies which are yet in the preliminary stage were carried out on white rats and in human subjects.

In the case of the animal experiments serum levels and urinary excretion of calcium and the level of blood phosphorus and its excretion in the urine over a period of 6 hours immediately following acute stress by a single subcutaneous injection of formaline were determined and compared with the non-stress levels. A simultaneous study of blood and urinary creatinine gave an estimation of the glomerular filtration from which an assessment of the phosphorus load and its tubular re-absorption could be computed. Re-absorption of phosphorus by the renal tubules thus determined helped to assess activation of parathyroids. Fall in circulating eosinophil count was used to establish the stressful situation in the animals, which was confirmed by adrenal enlargement in the animals that were sacrificed at the end of the collection of the blood and urine samples.

Parathyroids were also removed to study the alterations in their wet weight and changes in histology, particularly for evidence of hypertrophy.

Similar studies were carried out on 6 parathyroidectomized rats (40 days).

The study in human subjects consisted of examining the endogenous creatinine clearance and blood and urinary phosphorus levels and thereby assessing the renal tubular re-absorption of phosphorus before and over a period of 6 hours after stress of surgery. Six human subjects have so far been studied. While as pointed out above, the studies are still in the preliminary stage only, it appears that marked change in phosphorus levels of blood and its excretion occur immediately following stress and that this response is modified in the rats that have been parathyroidectomized previously.

The study is being continued on the above lines to collect more data regarding the changes occurring at different time intervals following stress and under different kinds of stress to see if the effects are truly of a non-specific nature and if the parathyroid activation is mediated, wholly or partly, through adrenal glands by using adrenalectomized animals also.

227. Investigation of gastric secretory function by tubeless method as compared with the standard intubation method under Dr. M.L. Pai at the Medical College, Baroda.

Fractional gastric analysis has been since long the method of choice for the examination of the gastric-secretory function. This method, however, has two major disadvantages. Even with expert hands, the introduction of the tube often proves distressing to the subject and the act of swallowing the tube or its retention in the stomach tends to alter the composition of the juice. Recently, the utilization of the cation exchange indicator 'Diagnex' has been suggested, as a method for determining the presence or absence of free hydrochloric acid in gastric contents without the need for gastric intubation. 'Diagnex' is produced by replacing the hydrogen ions of the carboxylic acid groups with quininium ions. In the presence of free hydrochloric acid, such as gastric juice, the hydrogen cation of the acid replaces the quininium cation releasing quinine, which is absorbed in the small intestine and approximately one-third is excreted in the urine. This test is based on quantitative estimation of quinine in urine.

The investigations on the Inquiry have been carried out on following lines. Subjects or patients belonging to different types of responses, namely achlorhydria, iso-chlorhydria and hyperchlorhydria as examined by alcohol and histamine meal tests have been investigated after the quantitative diagnex test and the results obtained after these two tests have been compared by the method standardized here (Pai, 1956).

Some preliminary work was done on eighteen subjects on the lines as suggested above and an attempt was made to see whether any quantitative relationship could be established between the gastric acidity as determined by the fractional gastric analysis method and the excretion of quinine in urine after the diagnex test. The results of this work have been published (Pai, 1956). Further work has been done on the

lines similarly followed as above. Nineteen subjects have been studied before treatment and out of these seven have been studied after treatment. The subjects have been selected, after the fractional gastric analysis method either after the alcohol meal or the histamine test meal, for the diagnex test. These are obtained with different types of responses, namely, achlorhydria, hypochlorhydria, isochlorhydria and hyperchlorhydria. The results of the study in the subjects made so far, both before treatment and after treatment, show that there is a quantitative relationship present between the level of the free acidity in the gastric juice and the level of urinary excretion of quinine after the diagnex given by oral administration (*vide* Table). This has got to be confirmed by further analysis of a larger group of subjects.

Comparison of highest free acidity and total quinine excretion.

Before treatment

Group	Number of subject	Average highest free acidity in cc. of N/10 HCl in 100 cc.	2-hours average total excretion of quinine after diagnex mg.
1. Achlorhydria	4	0	49.8
2. Hypochlorhydria	3	10.1	155.4
3. Isochlorhydria	3	35.2	189.4
4. Hyperchlorhydria	9	103.5	328.9

After treatment

1. Hypochlorhydria	2	10.5	132.2
2. Isochlorhydria	2	22.5	154.0
3. Hyperchlorhydria	3	75.2	373.8

228. Inquiry on the effect of distention of various parts of intestine (small and large) on circulatory, respiratory and alimentary functions under Dr. B.S. Kahali at the S.M.S. Medical College, Jaipur.

Fifty apparently healthy vagabond dogs supplied by the municipality were used as experimental animals. Urethane, nembutal and chloralose were used as fixed dose anaesthetics in a preliminary series of experiments. Morphine with urethane and nembutal were found unsuitable as they were found to depress some of the functions under investigation. All subsequent experiments were done under chloralose anaesthesia (60 to 70 mg./kg in 2 per cent solution in saline I.V.).

The second part of the duodenum was distended with air in a balloon attached to a catheter introduced into the lumen of the intestine by a linear cut into the antimesenteric border of the intestine. Three levels of pressure 10 mm., 20 mm. and 40 mm. of mercury were used. The rectum was similarly distended.

The blood pressure was recorded from the left carotid artery by the usual technique; the same kymographic record run on medium speed was used for counting the heart rate.

The respiration was recorded with a tambour attached to the tracheal cannula initially but later on with the automatic tidal volume recorder (Phipps and Bird, Inc.).

For recording movements of the various parts of the alimentary track, various methods were used: (i) Intraluminal air filled balloon tied to catheter or a Miller-Abbot's tube connected to an ordinary or a research air tambour (Phipps and Bird, Inc.). (ii) Intraluminal water-filled balloon tied to a rubber catheter. The catheter was connected through a Y-glass tube with a burette to which water can flow when contraction of the intestine occurs and with a water reservoir for filling the balloon. The upper end of the burette was connected with a tambour for recording intestinal movements. (iii) Jackson's enterograph and (iv) Cushny's myocardiograph.

The respiratory movements interfered with the record of the intestinal movements. A longer abdominal incision and raising and fixing the lateral flaps of the abdominal incision at a height of 1" to 2" above and filling the peritoneal cavity with warm Tyrode solution improved the intestinal record in all the methods.

Of the methods used, Cushny's myocardiograph, gave the best results with little or no interference by the respiratory movements. This method has been used in most of the experiments subsequently. For the rectal movement where Cushny's myocardiograph cannot conveniently be used, the improved water-filled balloon technique has been used.

The secretion was collected from various parts of the gut with a rubber catheter or a polyethylene tube with side holes introduced into the lumen of the gut and fixed with purse-string sutures.

Results.—The results can be summarised as follows :—

1. Duodenal and rectal distension caused cardiac slowing to the extent of 10 to 30 per cent of the initial rate and rise of blood pressure 5 to 20 mm. Hg. (5 to 20 per cent of initial level). This is possibly due to increased vasomotor tone.
2. Duodenal and rectal distension caused slowing of the respiration (10 to 20 per cent of the initial rate) with prolongation of inspiration. There was a rise of 5 to 20 per cent of tidal volume but the pulmonary ventilation per minute was diminished in most cases.
3. Duodenal distension caused increase in tone and rhythmicity of the jejunum, ileum, caecum, appendix, transverse colon and the rectum. The stomach showed diminution of tone and rhythmicity. The ascending colon was unaffected. The distension of the rectum caused increased tone and rhythmicity of the stomach, duodenum, jejunum, ileum, caecum and the transverse colon.
4. Duodenal distension caused increased secretion of duodenum distal to the site of distension rich in bile and of jejunum. The effect on the gastric secretion was variable while that of the ileum was unaffected. The caecum, ascending colon, transverse colon and the rectum did not show any secretory activity before or during duodenal distension. The distension of the rectum caused diminished secretion of the stomach, duodenum, jejunum and the ileum, while the rectum, usually dry, gave a slight mucoid secretion. The caecum, ascending colon gave and transverse colon did not show any secretion before and during rectal distension.
5. The circulatory, respiratory, motor and secretory responses were better marked with 20 mm. and 40 mm. than with 10 mm. of mercury pressure distension.
6. The circulatory, respiratory and motor effects to distension ceased with the cessation of distension but the secretory effects continued. The circulatory, respiratory and motor effects are presumably intermediated by nervous mechanism while the secretory responses may be through the hormonal mechanism alone or through both hormonal and nervous mechanisms.
7. The circulatory, respiratory and motor effects did not show any decrement during maintenance of distension, the receptors involved do not show any adaptation.
8. Presence of stretch receptors in the regions of the duodenum and the rectum have been established.

229. Studies of 17-ketosteroids output in health and disease of Indians under Dr. J. C. Sachdev at the M. G. M. Medical College, Indore.

DISEASE IN INDIANS.

The present work deals with the determination of 17-ketosteroids and gluco-corticoids excretion in the urine of normal healthy subjects. 17-Ketosteroid output was determined by the method as recommended by M.R.C. Committee on Clinical Endocrinology (1951) in sixty subjects between the age of 17 and 40 years. These subjects were selected from among medical students and the medical staff of the institution. A thorough clinical examination was undertaken and those with any suspicion of disease or history of illness, immediately before the investigation were excluded from the study.

It was observed that in 32 subjects aging 17 to 19 years the daily excretion of 17-ketosteroid ranged from 4.3 mg. to 27.4 mg. with a mean value of 13.46 mg. In another 14 subjects with age varying from 20 to 24 years, the average 17-ketosteroids excretion was 6.1 mg. to 25.6 mg. with a mean value of 12.42 mg. In the group of subjects 25 to 40 years old, daily 17-ketosteroid output varied from 7.63 mg. to 12.03 mg. with a mean of 10.86 mg.

The above study does not reveal any appreciable difference in 17-ketosteroid excretion in the subjects from 17 to 40 years but a slight decrease in the excretion of 17-ketosteroid was observed in group three when compared to group one.

Average values in these healthy subjects, so far investigated, are somewhat lower than those reported by the foreigners for their population but definite conclusion can only be drawn after the work has been completed.

Bio-assay method of Eggleston, Johnston and Dobriner for glucocorticoids in the urine using mice has been slightly modified and is being developed in this Laboratory.

Preliminary studies in normal groups of controlled animal (mice) which were adrenalectomized and received sesame oil without any urinary extract gave average figures of 0.7 mg. in terms of glucose.

230. Drug Research Unit under Dr. M. L. Gujral at the K. G. Medical College, Lucknow.

The antiarthritic property of *Glycerrhiza glabra* (mulethi) and the antiphlogistic property of the resinous gum of *Balsamodendron mukul* (guggul) have been studied employing the formalin-induced arthritis technique in rats in the former case and the 'granuloma-pouch' technique

of Selye in the latter case. Various extracts of the crude drugs, viz., watery, alcoholic and petroleum ether, have been tested employing hydrocortisone and butazolidine as control drugs for getting a comparative idea of the efficacy of the drugs under study.

The results, after statistical analysis of the data, indicate that alcoholic and petroleum ether extracts of 'guggul' have considerable antiarthritic and anti-phlogistic properties. Comparable results have been obtained with glyceric acid.

231. Drug research unit under Dr. C.L. Malhotra at the Lady Hardinge Medical College, New Delhi.

The work on *Herpestis monniera* was continued under the Drug Research Unit. In addition, studies were conducted on *Rauwolfia alkaloids*, indigenous plant *Withania somniferum* and certain aspects of hypothermia.

I. HERPESTIS MONNIERA

(1) *Chemical studies*.—Previously a non-active non-reducing sugar, *d*-mannitol was isolated in this laboratory. It was confirmed by mixed melting point and infra-red spectra. In addition, there was an indication for the presence of a glycoside-saponin fraction. It has now been possible to isolate a pharmacologically active substance in a pure crystalline form. It is colourless, crystalline, highly hygroscopic substance, M.P. 230°C, insoluble in water, acetone, ether, chloroform but soluble in methyl alcohol, 90% ethanol and ethyl acetate. However, it forms a stable colloidal solution with water in the concentrations used experimentally. It dissolves in sulphuric acid giving a golden yellow colour and foams heavily with water. In dilute solutions it stabilises an emulsion of kerosene oil in water. Further chemistry of this fraction is being worked out.

(2) *Pharmacological studies*.—The following investigations have been done with the pure crystalline pharmacologically active principle referred to above :—

- (i) *Frog's heart*.—When given in single doses in isolated perfused heart it had generally no action in doses of 0.05 mg. to 0.25 mg. In doses of 0.3 mg. to 1.0 mg., it generally caused a mild to moderate positive inotropic action, sometimes preceded by a transient negative inotropic action. In doses higher than 1.0 mg., it caused standstill of heart in diastole or midsystole. The drug had variable effect on cardiac rate. Frequently it caused tachycardia and sometimes bradycardia. Usually bradycardia was associated with negative inotropic action. No significant disturbance in rhythm was seen. In many frogs it produced imperfect diastolic relaxation with a reduction in systolic size indicating an increase in cardiac tone.

Isolated frog's hearts were perfused with frog's Ringer containing the pure crystalline active principle in concentrations ranging from 1×10^{-5} to 4×10^{-5} . In concentration of 1×10^{-5} it usually had no activity. In concentrations of 2×10^{-5} and 1.33×10^{-5} it generally had a positive inotropic action with either tachycardia, in some cases bradycardia or no action on cardiac rate. At this dose level cardiac irregularities in the form of A-V block were also noticed in majority of frogs. In concentration of 4×10^{-5} it mostly had a negative inotropic and negative chronotropic responses which could be reverted to normal on changing to plain Ringer perfusion.

- (ii) *Rabbit's heart*.—On the Langendorff's preparation of rabbit's heart in single doses of 0.5 mg. to 1.0 mg., it had either no effect or a negative inotropic and negative chronotropic actions. No cardiac irregularity was observed.
- (iii) *Guinea-pig*.—In guinea-pigs the pure crystalline principle was given intravenously at the rate of 1 mg./kg./min. for 25 minutes and thereafter at the rate of 2mg./kg./min. for another 10 minutes. E.C.G. was recorded. A moderate degree of bradycardia was observed from 10th minute onwards. There was a significant increase in P—R interval and prominence of T wave from 15th minute onwards. There was no cardiac irregularity. E.C.G. changes and cardiac rate returned to normal 25 minutes after stopping the infusion. An interesting observation was that though the cannulation of the external jugular vein was done under light ether anaesthesia only in the beginning, no further anaesthesia was required throughout the period of infusion (35 minutes). Throughout this period the animal remained anaesthetized, corneal reflex was absent and respiration was slow. Thirty-five minutes after stoppage of infusion, the corneal reflex returned and respiration became hurried. The animal began to respond to painful stimuli after another two hours.
- (iv) *Albino rats*.—In a few experiments conducted on albino rats, the crystalline principle was found to have a hypnotic effect.
- (v) *Frog's rectus and gastrocnemius muscles*.—The crystalline principle had no effect on frogs's skeletal muscle. The whole extract, however, had a marked spasmodic effect on the rectus muscle which was not easily washed out. The whole extract has also been found to have anti-acetylcholine and neuromuscular blocking actions.

II. RAUWOLFIA SERPENTINA ALKALOIDS

The following investigations have been done with the pure alkaloids of *R. serpentina*.

- (i) *Anti-nicotinic action*.—It was found that the alkaloids of *R. serpentina* and isonicotinic acid hydrazide abolish the nico-

tine induced tremors in rabbits and thus have central anti-nicotinic actions.

- (ii) *Reserpine on acetylcholine and glutathione content of brain.*—Acetylcholine and glutathione contents of various areas of brain have been estimated with particular reference to hypothalamus, in normal untreated dogs and in dogs which have been administered reserpine.
- (iii) *Reserpine on blood pressure responses evoked from limbic system.*—Previous work showed that reserpine not only depresses the sympathetic centres but also facilitates parasympathetic centres in the hypothalamus. This work is being further continued by implanting electrodes in the various other areas of the limbic system and noting the effect of reserpine on blood pressure responses evoked from these areas.

III. WITHANIA SOMNIFERUM

Preliminary studies with the watery whole extract of the root of *Withania somniferum* were conducted.

A. Dog :—

- (i) *Cardio-vascular system.*—The extract when given intravenously in anæsthetized dogs produced a moderate to marked degree of hypotension of acute onset and of variable duration lasting from 5 to 30 minutes depending upon the dose administered which was completely blocked or markedly reduced by atropinisation. There was increase in spleen volume. It markedly reduced the pressor response to nicotine bitartrate but appears to enhance in degree and duration the pressor response to epinephrine. It blocked the carotid baroreceptor reflex.

The extract had insignificant effect on cardiac rate in small doses but in high doses it produced mild degree of bradycardia.

- (ii) *Respiratory system.*—In anæsthetized dogs intravenous administration of the extract produced mild to moderate degree of stimulation of rate and depth of respiration. The respiratory action was independent of cardio-vascular action as it could be elicited even after atropinisation (when there was no hypotensive response). It also did not block respiratory stimulating effect of nicotine.
- (iii) *Intestinal movements.*—The extract increased the amplitude of movements and tone of intestinal muscle in intact anæsthetized animals.

B. Frog.—

- (i) *Heart.*—Preliminary studies on the perfused frog's heart showed that it caused an immediate temporary diastolic standstill or marked negative inotropic and negative chronotropic action followed by marked positive inotropic and positive chronotropic action.

- (ii) *Central nervous system.*—In small doses it had a tendency to cause some excitement but in higher doses it caused neurological deficit and still higher doses were fatal.

C. Albino rats.—

Preliminary experiments in albino rats showed that the extract had a hypnotic effect.

IV. HYPOTHERMIA

In dogs hypothermia was produced by veneshunt through ice and surface cooling. After the temperature came down to 10°C. to 12°C. the heart and different parts of brain were taken out and their acetylcholine and glutathione content estimated. The results indicate a decrease in acetylcholine and glutathione content of heart and hypothalamus after hypothermia. However, there was an increase of both in the temporal lobes containing Ammon's horns.

232. Inquiry into the antidiabetic properties of some select indigenous plants under Dr. Ranita Aiman at the B. J. Medical College, Poona.

DRUGS INVESTIGATED WERE :

- (i) *Pterocarpus marsupium.*
- (ii) *Bassia longifolia.*
- (iii) *Casearia esculenta.*

The studies reported previously were continued to determine if these drugs exert any action on the fasting blood sugar of normal animals or animals made diabetic by alloxan or pancreatectomy.

Animals used were rabbits and dogs. The drugs were used as cold (C.W.E.) and hot (H.W.E.) water extracts and tried in 2 doses.

FASTING BLOOD SUGAR (F.B.S.) OF NORMAL RABBITS.—The drug was given by stomach tube to animals fasted 18 hours and blood sugar (B. S.) estimations were done before, and 1/2, 1, 2, 3 and 4 hours after drug administration. The results were compared with control values obtained by administering distilled water in the same volume and way; the value for the first blood sample being taken as 100 in all cases.

ALLOXAN DIABETES :

Rabbits :— Alloxan monohydrate 180 mg./kg. body-weight was given to drug-treated rabbits, the drug being given two hours before the I. V. injection of alloxan and daily after it. B.S. estimations were done before, and 1/4, 1/2, 1, 2, 4, 6, 8, 24, 48, 72, 96 hours after alloxan. Two rabbits used as controls were given only alloxan and no drug and B. S. studies were done as described above.

Dogs.—Alloxan monohydrate 60 mg./kg. body weight was given I.V. to dogs treated with the drugs in the same way and B.S. studies were also done as described above for rabbits. The dogs were observed for a longer period of time. The B.S. values were compared with control alloxan diabetic dogs.

All animals were given fixed weighed diet.

DEPANCREATISED DOGS.—Dogs were given the drug daily for varying periods to see if it affected the F.B.S. in any way, and a dextrose-tolerance curve was also done. They were then operated, and the drug again given. B. S. estimations were done frequently and insulin given to control partly the intensity of the diabetes and maintain the fasting B. S. at a constant level, as far as possible. A dextrose-tolerance curve was also done. The drug was given for about 30 days. All animals were given a fixed weighed diet.

PTEROCARPUS MARSUPIUM :

F. B. S. of normal rabbits.—Eight rabbits were used, 6 tests were carried out with the H.W.E. and 6 tests with the C.W.E in 2 doses. No hypoglycæmia was observed.

Alloxan diabetes : Rabbits.—C. W. E. was tried on 3 rabbits. One animal died 30 hours and one after 50 hours. The other was sacrificed on the 5th day and the organs examined histologically.

Dogs.—Two dogs were used. One was observed for 4 months. The F.B.S. estimations repeated frequently during this period fluctuated between 130 mg. and 193 mg. per cent. The other dogs died on the 7th day. Diabetes was severe

Pancreatectomy.—Two dogs were studied. No evidence of an action in diabetes induced by alloxan in rabbits or pancreatectomy in dogs was obtained.

BASSIA LONGIFOLIA :

F.B.S. of normal rabbits.—Ten rabbits were used. Nine tests were carried out with the H.W.E. and 5 tests with C.W.E. in 2 doses. No hypoglycæmia was observed.

Alloxan diabetes : Rabbits.—C.W.E. was tried in 7 rabbits. Two animals died 6 to 8 hours after alloxan, one on the first night and another the second night. The other were sacrificed on the 5th day and the organs examined histologically. From the data, it appears that blood sugar in the 24, 48, 72, and 96 hours blood samples are lower than the control figures when the first sample in both groups is taken as 100.

Dogs.—Two were studied, one for 2 months during which time F.B.S. estimations were done, and the other 8 days. The former had to be given insulin and the dosage increased owing to a rising B.S.

Pancreatectomy.—One dog was studied for 32 days. From the above studies it appears that the drug had no effect on diabetes in dogs induced by alloxan or pancreatectomy.

CASEARIA ESCULENTA :

F. B. S. of normal rabbits.—C.W.E. was given to 2 rabbits in the same dose. The 3rd and 4th readings were lower in the drug treated group as compared to control values.

PHYSIOLOGY OF HUMAN REPRODUCTION

233. Studies on twins and consanguinity under Dr. L. D. Sanghvi at the Indian Cancer Research Centre, Bombay.

Study on twins.—One hundred and twenty-seven twin pairs have been investigated so far. Information was collected under five major categories : (i) personal history, including scholastic performance; temperamental differences of twins; history of any major illness and congenital marks and defects, (ii) continuous morphological traits, (iii) discontinuous morphological traits, (iv) genetical traits, and (v) family history of twinning. In addition, photographs and finger prints were recorded in all cases.

A preliminary analysis was carried out on 100 twin pairs with regard to zygosity. It was possible to separate 50 twin pairs as dizygous on the basis of sex and genetical characters, which included (i) A_1A_2BO blood groups, (ii) MN blood groups, (iii) Rh factor with sub-types, (iv) taste reaction to phenylthiocarbamide, (v) secretion of ABH antigens in saliva, and (vi) colour vision. Fifty pairs still remained which were alike in sex and behaved similarly in all the above genetical traits. Seven pairs, out of these 50, were subsequently classified as dizygous on the basis of finger print analysis. Of the remaining pairs, 35 were classified as monozygous and 8 were left out as doubtful as no definite decision regarding their zygosity could be reached on the basis of available data. More work is being done for the refinement of criteria for the diagnosis of zygosity.

Study on consanguinity.—Rates of consanguineous marriages have been studied in fifteen endogamous groups in Bombay with a sample of about 500 marriages for each group. Ten of these groups belonged to the Marathi speaking Hindus, three belonged to the Gujarati speaking Muslims and the remaining two, to the Parsi and Christian communities. The rates of consanguineous marriages among the Marathi speaking Hindus varied from 3.5 to 11 per cent of which the bulk was made up of the first cousin marriage of the type of a boy getting married to his maternal uncle's daughter. The three groups of Muslims showed high rates of consanguineous marriages : Memans, 27.1 per cent; Bohras, 26.0 percent ; Khojas, 13.0 per cent, Parsis also showed a high rate of 17 per cent. Christians showed comparatively lower rates. All the four types of first cousin marriages were common among the Muslims, Parsis and Christians.

234. Investigation to study the function of ovary following hysterectomy, under Dr. P. C. Das at the Eden Hospital, Medical College, Calcutta.

In the present study a total of ninety selected cases has been studied. Of these, 50 cases belong to (1) hysterectomy group, and the remaining forty cases as (2) control not having hysterectomy. In the hysterectomy group, operation was performed for the following reasons :—

	Cases.
1. Fibroid of the uterus	24
2. Procidentia with some pathology of the uterus	14
3. Metropathia hæmorrhagia	5
4. Tubo-ovarian mass with extensive adhesion with the uterus	4
5. One case of rupture of the uterus during difficult labour	3

Six cases of subtotal hysterectomy are included in this series.

The age of the patients in this group varied from 22 to 56 years. Twenty-three cases below the age of 40 years, nineteen cases between 40 and 45 years and eight cases above the age of 45 have been studied. In sixteen cases, unilateral oophorectomy was done along with hysterectomy.

Among the control group are included :

(i) Normal non-pregnant women (ii) patients who had plastic vaginal operations, and (iii) one case of unilateral ovariectomy. The age of the control group varied from 20 to 45 years.

METHOD OF STUDY.

(A) Investigations were based on vaginal cytology, and (B) estimation of pregnanediol in 24 hours urine.

A. Vaginal cytology consisted of study of vaginal epithelial cells along with other constituents of the vaginal secretion. This was done by examination of the vaginal smears by the following process.

- (i) Collection of vaginal secretion from the posterior fornix by aspiration and its deposition on a clean glass slide.
- (ii) Fixation of the sample of smear.
- (iii) Staining of smears by modified Shorr's stain.
- (iv) Microscopical examination of stained vaginal smear.

The method of de Allende and Orias has been followed.

B. Estimation of pregnanediol in urine was done as described by Sommerville, Marian and Keller (1948).

Report of vaginal cytology.— Periodic vaginal smear examinations were done both pre-operative and post-operative to hysterectomy. Vaginal cytology before operation corresponded with the menstrual cycle. After hysterectomy periodic smear examination was done starting from one week after operation upto the longest period of five months. Most of the cases showed active smears following hysterectomy. Percentage of cornification varied from 8 per cent to 80 per cent, in patients below 40 years of age, from absence of cornification to 50 per cent in cases between 40 and 45 years and 14 per cent to 31 per cent in cases above 45 years.

Absence of cornification was found only in two cases within two weeks to one month after hysterectomy although one ovary was retained in one patient and both the ovaries in the other patient were preserved. Constant low level of cornification were found in four cases. In other forty six cases, fluctuant level of cornification was detected. Constant high level of cornification was not found in any case. It was also observed that cells of the deeper layer of the vagina appeared in the vaginal secretion as early as one week after hysterectomy cases at earlier age group. No definite relation could be found out with the percentage of cornification and percentage of cells of deeper layer in the vaginal smear. Percentage of deeper cells in the vaginal smear varied from very few in number up to as much as 44 per cent, larger numbers found in the older age group, i.e. after the age of 40.

In the control group, we have examined 40 cases. They were followed up to the maximum period of two months. Percentage of cornification varied from 5 per cent to 88 per cent. Most of the smears corresponded with the menstrual cycle. Absence of secretory phase was found in 8 cycles belonging to eight different patients. Of these 8 patients, high level of cornification was found in 3 cases and persistent low level of cornification in two cases. Cells of the deeper layer were found only in seven smears one going up to 10 per cent.

B. Estimation of pregnanediol in urine.— Routine estimation of pregnanediol in urine in all the cases were not possible. Among the hysterectomy group of cases, pregnanediol excretion in 24 hours urine varied from 0.128 mg. to 4.5 mg. Only in three cases pregnanediol excretion was above 2 mg. in 24 hours sample. In the rest pregnanediol was detected in minute quantities.

In the control group maximum pregnanediol excretion was 6.5 mg. in 24 hours sample during luteal phase.

SUMMARY.

1. It would appear that ovary continues its function and fairly normally after hysterectomy up to five months, the maximum period of follow up in this series.
2. Ovulation occurs in some cases, in this series only three as proved by both vaginal smear and pregnanediol estimation in urine.

235. Inquiry on radiation hazards affecting fertility under Dr. S. P. Basu and C. L. Mukerji at the School of Tropical Medicine, Calcutta.

Experimental studies on the 'radiation hazards' affecting fertility were started on a series of golden hamsters. To start with, eight pairs were irradiated as follows : Four pairs on gonads, three pairs on pituitary and one on general body surface. A few pairs were kept as control to watch the rate of breeding dependent on climate or dietary variation. Each animal weighed 100 g. and age was three months. All the pairs had litter before radiation was started. Radiation varied from 24 r to 144 r excepting the pair for general surface radiations, where the total r output at the source was 2,238 at a distance of 3 ft. Seven pairs having radiation on gonads and pituitary were separated for about 3 weeks after which they were reunited. Two pairs out of four for gonads had no litter for six to nine months, while for the other two, one had after 5 months and another after 6 weeks. The pair keeping sterile for nine months had a dose of 24 r. Those having radiation on the pituitary had litter, the shortest time being four weeks and the longest 15 weeks. Those two pairs, who have been sterile for a period of 6 to 9 months have been crossmated in order to determine the effect on the particular sex. The subsequent pairs, who kept sterile for $3\frac{1}{2}$ months, were again mated with normals who had no radiation. The result is being watched. Siblings born to irradiated animals have been crossmated to note the effect on the 2nd generation and mutation will also be watched. Mature siblings of irradiated animals have been mated with normal one, born to control group at the same time.

Vaginal smears were examined in an irradiated female animals with the following results :

Pituitary exposure	{	Percentage of	carnified cells	61·0
		„ „	basal cells	0·8
Ovary exposure	{	„ „	carnified cells	12·5
		„ „	basal cells	19·0
General body exposure	{	„ „	carnified cells	41·0
		„ „	basal cells	9·0

Comments :— The observations made on the small series of animals are not sufficient for any definite conclusion and the studies are in progress with groups of animals in several other series. Crossmating of animals with combination of irradiation sites are being done. From the results it appears that radiation on gonads may affect fertility. Irradiation of the pituitary appeared to cause no harmful effect on fertility.

236. Endometrial study in menstruation under Dr. S.C. Bose at the Medical College, Calcutta.

The object of the inquiry is to study the endometrium in anovular menstruation and its clinical correlation to physiological menstruation, sterility and dysfunctional uterine hæmorrhage.

Cases have been studied mainly from a series of cases complaining of sterility and irregular vaginal bleeding along with cases having normal menstruation to act as a control for the study.

Seventy cases have been studied so far and these include 55 cases of sterility and 15 cases of dysfunctional uterine bleeding. Endometrial biopsy revealed a non-secretory type of endometrium in 15 cases of sterility and 9 cases of dysfunctional bleeding. Cervical arborization of fern tree appearance was seen in 12 cases of the whole series. Vaginal smear study done in 42 cases revealed progesterone effect in 21 cases, good oestrogen effect in 18 and slight oestrogen effect in 3 cases.

A drop in temperature with a subsequent rise approximately at the same time in 3 different cycles was noticed in 28 cases.

Insufflation test done in 38 cases of sterility was negative in 6 cases of these 6 negative cases hysterosalpingography could be done in 3 cases and 2 revealed the blockage of tubes. Husbands semen examination done in 39 cases revealed some form of abnormality in 24 cases.

It appeared from the correlation of the data that of the total 70 cases so far studied about 22 were having anovulatory menstruation. They are under more detailed study as regards pregnanediol excretion values and abnormal endometrial patterns.

VIRUS DISEASES

237. Rabies enquiry under Dr. A.K. Thomas at the Central Research Institute, Kasauli.

1. Twenty strains of locally isolated street virus and one strain of fixed virus were tested in tissue culture (monkey kidney cells) for evidence of virus multiplication. None of the strains thus studied showed any evidence of virus multiplication when sub-cultured or inoculated into mice intracerebrally.

2. Extract of Arachis oil and egg yolk were tried for the prevention of experimental encephalomyelitis in guinea-pigs. Neither the extract of Arachis oil nor the extract of egg yolk was able to save life or prolong the incubation period in guinea-pigs suffering from experimental encephalomyelitis.

3. Purification of rabies vaccine by methanol-chloroform mixture and bentonite was attempted. Bentonite purified vaccine showed a 10-fold loss of virus.

4. Experiment on the quantity of anti-rabic vaccine and duration treatment on immunity shows that an efficient protection can be obtained with a smaller dose of vaccine, given daily for a longer period.

238. Polio Research Unit under Dr. P.V. Gharpure at the Grant Medical College, Bombay.

A. *Epidemiological*.—Poliomyelitis in Bombay has been again studied this year. The incidence of paralytic cases, their location, age-distribution, deaths, types according to paralysis, seasonal distribution, etc., have been studied. 189 cases were recorded. The mortality is about 10 per cent. Study done in Greater Bombay has shown that there has been no change in the pattern of the disease. The age of onset has been the same, *viz.* the bulk of the cases are below 3 years and other features studied indicate that the incidence is not rising. There is a steady occurrence of cases in all parts of the town and its suburbs. The sera studied confirm that in age group above 3, neutralizing antibodies develop in a large proportion of persons. Analysis of the population sample shows that of 10,000; 654 are between 6 months and 3 years, 144 are below 6 months. The average births from 10,000 can be stated as approximately 100 per year. Should a vaccination scheme be thought of, these figures will help to assess the size of the project.

Studies were commenced in Dohad. The work so far done mainly has been to collect data and materials. Material for virus isolation has been dealt with. Sera from age-group 5 to 12 have been collected and tested.

Material received from Dr. Seal from Andaman and Nicobar Islands has been studied. These studies show that poliomyelitis that occurred in those Islands in the year has been more or less on the same pattern as that in India. Polio virus was isolated. The typing of the virus has been in hand. Owing to some scientific difficulty the final typing has been delayed.

Sera from young males were received from Lt. Colonel S.L. Kalra of the Armed Forces Medical College, Poona. These are from young men from all over India. The object of these tests was to get a direct proof of the population having come in contact with polio viruses. These data are presented Statewise.

B. *Virus isolations and virus studies*.—780 samples of stools were tested for viruses in tissue cultures; 65 viruses were isolated: of these 11 are polio virus type 1, 3 are type 2 and 2 are type 3. Nine are not polio viruses on serological grounds and 40 are yet to be defined. The polio viruses have been defined only on serological grounds and their pathogenicity to monkeys is yet to be studied. The nine non-polio viruses are being studied. A beginning has been made to study the pathogenicity to suckling mice. Type specific sera for these and other non-polio viruses with the Unit have been undertaken. A set of sera have been kindly supplied by Dr. Melnick. These will help the study.

In 1949-55 all polio viruses obtained were by monkey inoculations and there was no possibility of detecting non-pathogenic or less virulent strains. The relative virulence of strains isolated so far will be an

important matter and work has been commenced on these. Of the other viruses isolated since tissue cultures were used for isolation, many viruses have created new problems and a scheme for their study has been under consideration. Of particular interest are the polio viruses obtained from contacts and healthy children. Over a dozen such strains are with the Unit. The study of these are likely to show presence of avirulent strains. There is a large group of viruses which on serological grounds are not polio-viruses. There are 63 such strains. The question of their identity has been taken up.

C. *Serological studies*.—During the year under report 1,146 sera from different sources were received. Of these 500 have been tested. One group (212) coming from adults from all parts of India were supplied by Lt. Colonel S.L. Kalra. In these sera only two have shown complete absence of neutralizing antibodies against all the three types of the polio virus.

The other group, 31 sera are from children in Bombay. In these there is evidence of immunization of children above the age of 3. 122 sera collected in Dohad were tested. These were from age-group 5 to 12 years. These show a high percentage of immunized children. 225 sera were received from Dr. Seal. These had been collected by him in Andaman and Nicobar Islands. Of these 100 per cent from ages below ten, almost 100 per cent from age group 10 to 15, and 50 per cent of the higher age groups were selected for testing.

Analysis of the results shows that the process of immunization in nature noticed in these Islands is the same as seen today in India. Earliest, there is immunization against types 1 with progressive immunization against types 2 and 3 and by 10 years almost 100 per cent show neutralizing antibodies against all the three types. Nicobar Islands which in 1947 was a virgin soil is no longer so today. These facts form an epidemiological point of view are worth noting. The Unit devoted much time for the investigations and the results show that the trouble has been worth taking.

239. Inquiry on the incidence, nature and types of coxsackie virus infections in Bombay City under Dr. A.N.D. Nanavati at the Seth G.S. Medical College, Bombay.

1. *Collection of material*.—Fæcal samples from 34 poliomyelitis patients admitted at Bai Jerbai Wadia Hospital for children, Bombay, from 33 normal children admitted at the K.E.M. Hospital, Bombay, for orthopædic operations and from 33 cases of influenza admitted at the Arthur Road Hospital, Bombay, were collected to incriminate strains of Coxsackie viruses by routine methods of new-born albino mice inoculations.

A collection of 28 autopsied brain and/or spinal cord samples from suspected cases of encephalitis, etc., was made to recover, if possible, any other mouse adaptable virus, thus making a total of 437 cases from the beginning of the work on the virus investigation.

2. *New-born mice inoculations.*—Processed fæcal samples from 30 poliomyelitis patients and 33 normal children were investigated for the recovery of Cocksackie viruses by subcutaneous inoculations into new-born albino mice. Three positive isolations, one from the fæces of poliomyelitis patient and two from the fæces of normal children, were made. All the three strains are being studied by neutralization tests into new-born mice with known typed antisera for their antigenic pattern.

3. All the twenty-eight brain and/or spinal cord samples gave negative results on an attempt to isolate any mouse-adaptable virus by intracerebral inoculations into adult (4 to 6 weeks old) albino mice.

4. *Tissue culture studies.*—(a) Attempts were made to grow the embryonic mouse and chick heart and lung tissues on plasma clots with different nutrient media. Experiments were also carried out to trypsinize embryonic mouse and chick tissues after beheading them. Ten of the locally isolated strains belonging to group A types 1, 3, 4, 10, 12, 14 and the remaining four untypable strains of Cocksackie viruses were passaged into the respective embryonic mouse and chick tissues. None of the strains under study, gave any signs of cytopathogenesis up to the 3rd passage into the respective tissues. The harvested fluid after the third tissue culture passage failed to induce signs of illness into new-born mice after receiving subcutaneous inoculations of the fluid, indicating the absence of multiplication of the strains under study.

(b) Ten of the above-mentioned strains, five of the locally isolated strains of orphan viruses and one strain of Cocksackie virus (Grigg strain) adaptable to monkey kidney cells (kidney made available to us by Dr. J.L. Melnick) were studied in a cell line of mouse fibrosarcoma (kindly supplied to us by the Department of Tissue Culture, Indian Cancer Research Centre, Bombay 12). The results of these studies are awaited.

(c) Processed fæcal samples from 15 cases of poliomyelitis were inoculated each into a pair of tubes of HeLa cells kindly received from Department of Tissue Culture, Indian Cancer Research Centre, for the recovery of either polio, Cocksackie (group B) or Orphan viruses. No positive isolations could be made from these samples.

240. Rabies inquiry under the Director, Pasteur Institute, Coonoor.

Last year we reported that it was not possible to demonstrate any protection among guinea-pigs challenged with the NYC or the Marimuthu strain of street virus and treated with serum before or after infection. Antirabies serum alone, while definitely prolonging the incubation period, had no saving effect with the challenge doses employed. The only condition under which serum seemed to confer some protection was when it was obtained from animals of the same species which had survived an infection with the particular challenge virus. This finding would suggest that serum homologous to the strain and species alone might be of value. Obviously, this condition is impossible to obtain in practice.

I. ANTIRABIES SERUM.

1. *Specificity of antirabies serum.*—In order to determine whether serum homologous to the strain alone would give protection, the value of serum (PIC) raised in horses against the Paris strain of rabies fixed virus as well as Lederle's serum, against an intramuscular challenge of 4 and 8 LD₅₀ of the Paris fixed virus, was investigated. It was found that the protection was the best among animals given the serum homologous to the strain.

Studies were then undertaken to determine the protective value of various antirabies sera against different strain of street and fixed rabies virus.

The sera employed were : (i) Lederle's serum, (ii) serum raised in horses against fixed virus, (iii) serum raised in guinea-pigs against fixed virus, (iv) serum raised in sheep against a fairly fixed strain of street virus obtained from Jackal 298, and (v) serum raised in guinea-pigs against Jackal 298 virus. The protective value of the above sera was tested in guinea-pigs against the following strains of virus : (i) NYC, (ii) Marimuthu, (iii) Masuli, (iv) Jackal 298, and (v) fixed virus.

It was found that when the above sera were given, there was no evidence of protection against the NYC, Marimuthu and Masuli strains of street virus. The results were good when serum homologous to the challenge virus was administered. The protection was the best when serum raised in guinea-pigs against the homologous strain was given. These findings confirm our earlier observation that for serum alone to give the best results it should be homologous to the strain of virus as well as the species of animal to be treated.

2. *Attempts to dissociate virus from serum-virus mixtures in vitro.*—Experiments were carried out to determine whether the virus could be dissociated from serum virus mixtures by (i) inoculation peripherally immediately after mixing, (ii) dilution, and (iii) administration of Cortisone. The results indicate that when serum and virus are mixed *in vitro* the union is firm.

II. COMBINED SERUM AND VACCINE THERAPY.

In our earlier experiments using a dosage of vaccine corresponding to the human treatment and Lederle's serum, it was found that the best results were obtained when one-fifth the dosage of serum recommended, *i.e.*, 0.25 c.c. per pound of body-weight, was employed. The results were poorer with larger as well as smaller doses suggesting that an optimum relation between the dosage of serum and vaccine had to be maintained for good results. It was also found that when vaccine alone was used for treatment better results were obtained when the 2.1 c.c. was administered in 14 instead of 7 doses. Further, it was possible to save some animals even when vaccine therapy was started post-infection provided the quantity of vaccine administered was increased about three-fold.

An experiment was undertaken in guinea-pigs to confirm the above findings and also determine whether with different doses of Lederle's serum it was possible to reduce the quantity of vaccine. The results indicate that (i) there is an optimum ratio between the dosage of serum and vaccine given, good protection being obtained with 0.25 c.c. of a 1 in 5 dilution of Lederle's serum and a total quantity of 2.1 c.c. of vaccine for a guinea-pig, (ii) the results were the best when the 2.1 c.c. of vaccine is administered in 14 equal doses of 0.15 c.c., and (iii) results some what comparable with combined therapy can be obtained with a large dose (7×1 c.c.) of vaccine, even when the treatment is started post-infection.

In an experiment to determine the protective value of 0.25 c.c. of a 1 in 5 dilution of Lederle's serum and 7×0.3 c.c. of vaccine, both being started 1 hour after infection, it was found that the survival rate was 40 per cent in guinea-pigs against a challenge of 15.7 LD₅₀ of NYC virus, while there was no protection against higher challenges, viz., 20.8, 26.2 and 31.2 LD₅₀.

In our earlier work using vaccines prepared from sheep brains varying in virus content, keeping the serum dosage constant, it was found that the best result was not obtained with vaccine prepared from the brain of a sheep whose virus content was the highest. Experiments carried out during the year confirm our previous finding that the optimum dosage of serum does not depend only on the dosage of vaccine and that it may have some relation to its antigenicity.

Using the same batch of vaccine it is found that when one-fifth the recommended dose gave good protection with Lederle's serum, the best results were obtained with 1/30th the dosage when serum prepared at the Institute is employed. It would, therefore, seem essential that the optimum dosage of each brand of serum in relation to the vaccine used has to be determined before embarking on serum therapy. Otherwise we will be doing more harm than good.

III. SERUM AND VACCINE PROPHYLAXIS IN HUMAN BEINGS.

Based on the above studies combined treatment with serum and vaccine is being given at the Institute to patients severely exposed to the risk of infection. The dosage of serum (PIC) given is 0.01 c.c. per pound of body-weight which is 1/25th the recommended dosage of 0.25 c.c. Among the 15 patients treated so far there has been no death.

241. Maintenance of stock cultures under Dr. K.J. Ranadive at the Indian Cancer Research Centre, Bombay.

The scheme on maintenance of stock cultures started functioning on 1st July, 1956. Its main objects are :

1. To maintain duplicate stock cultures of normal and malignant commercial cell lines for the Virus Research Centre, Poona, and to

supply on demand for virus research, the cell strains to different laboratories in India.

2. To carry out simultaneously the basic cytological and experimental studies on various normal and cancer cell types.

3. To develop new cell strains of various origins which are not available from commercial sources.

Method.—For stock, the cells are continuously cultivated on glass surface of 4 oz. neutral glass flat bottles. The culture bottles are incubated at $37^{\circ} \pm 1^{\circ}\text{C}$. Roller and stationary tubes with glass-slips and hanging drop preparations are used for experimental purpose.

Medium.—The nutritive medium consists of 'Basal Medium Eagle' (BME) supplemented with human adult serum or horse serum. Stock bottles take 15 c.c. of medium each which is renewed every 3rd or 4th day. Some cell strains are transferred every 7 days, others every 10 days. A minimum of 4 stock bottles per strain, two for sublines and 8 to 10 bottles for the experiments are maintained.

CANCER AND NORMAL CELL STRAINS

1. *Hela (cancer cell strain).*—The stock was obtained in March, 1956. It is maintained on BME supplemented with 10 per cent human serum and transferred on every 7th day. After 20 and 50 generations on human serum, two sublines have been developed on horse serum. They are in excellent condition and are in their 80th, 60th and 30th passages, respectively.

2. *KB (cancer).*—It was obtained in July, 1956, and has been kept on BME with 15 to 20 per cent human adult serum and transferred every ten days. At present this strain is in its 40th passage.

3. *Intestine (normal).*—It was obtained in July 1956. It is maintained in the same way as KB. It is in its 40th passage.

4. *Hell (cancer) and 5 Conjunctiva (normal).*—These two lines were brought in March, 1957. They are maintained on BME supplemented with 20 per cent human serum. They are transferred every 10 days. They are in excellent condition in the 20th passage.

6. *Det. 6 (cancer).*—It was obtained in March, 1957. This requires higher percentage (25 per cent) of human adult serum with BME. It is transferred every 10 days. Now it is in its 22nd passage.

In addition to the above maintenance of stock cell strains, attempts are being made to develop a cell line from human fibrosarcoma as this cell type is not commercially available. One line of epithelium, like cell strain (HLS₂) from human liposarcoma has been developed, which is now more than six months old and has established characteristics of a commercial cell line. Two human embryonic, one mouse embryonic and one mouse fibrosarcoma fibrocyte lines are being developed with the same technique. Basic cytological studies on these cell types are in progress.

242. Trachoma Pilot Project at the M.U. Institute of Ophthalmology & the Gandhi Eye Hospital, Aligarh.

TRACHOMA PILOT PROJECT

Conscious of the fact that trachoma and associated infections of the conjunctiva are responsible for the largest percentage of the blind in India, a two-year long trachoma pilot project was established by the Government of India with the technical advice of W.H.O., at Aligarh in the month of October, 1956. The Gandhi Eye Hospital and M.U. Institute of Ophthalmology, Aligarh, formed the base of this project which has the following objectives :-

1. To study in rural population the incidence and pattern of trachoma and the underlying factors favouring the transmission of infection;
2. To study the minimum effective course of antibiotic treatment for the Indian type of trachoma and the rates of relapse and of re-infection during the follow-up period;
3. To study the effect of intermittent treatment with antibiotics on the epidemiological aspect of associated conjunctivitis and the clinical picture of trachoma;
4. In the light of data and experience so gained to develop a programme of mass control which is effective, practicable and economically feasible.

Area covered.—29 villages with a total population of 22,000 in Jawan and Tappal I.D. blocks of District Aligarh, were covered.

Staff.—An Administrative Director, two ophthalmologists, one bacteriologist, one statistician, two socio-economic Lady Health Investigators, one health educator, fourteen dressers (theraputists) and other auxiliary staff were engaged. A W.H.O. Senior Medical Officer acted as an adviser.

During the first year of the project, though treatment was instituted for getting better cooperation, the main stress was laid on the incidence and pattern of trachoma and the underlying factors favouring the transmission of infection.

GENERAL SYSTEMATIC SURVEY

House to house, socio-economic, ophthalmological and bacteriological examinations were done.

(a) *Socio-economic investigations* indicate that most of the population covered presented a stereotyped standard of living which is very poor. The following items under study were applicable in a large majority : (1) One living room per household and average of 5 persons living in each household. (2) Poor economic level. (3) Water supply from the community well. (4) Cooking in open stoves (chulahs) just

outside the living room under the same roof. (5) No consumption of soaps or other detergents in the households. (6) Absolute lack of toilet facilities. (7) Commonly present illiteracy. (8) Habit of occasional bathing. (9) Utter lack of wiping facilities. (10) Habit of applying kajjal (eye-cosmetic) is almost universal. (11) Working place is either dusty or smoky.

(b) *Ophthalmological investigations* have revealed that the incidence of trachoma is from 80 to 90 per cent with 20 to 30 per cent of cases showing signs of trachoma (stages I, II and III). Usual age of onset is between 1 and 3 years. In babies less than one year, nearly 90 per cent are trachoma-free. Generally speaking, trachoma II is prevalent in age group 3 to 5 years, trachoma III in 6 to 8 years, and trachoma IV is maximum in adults. Spontaneous cure is not an uncommon feature. Only about 15 per cent of the conjunctival scrapings taken from trachoma I and II cases showed positive H.P. inclusions (Giemsa stain used). Females suffer from more protracted form of trachoma, showing higher figure of complications and loss of vision. The span of trachoma III is longer in females than in males. Associated conjunctivitis is common in children of pre-school age and the commonly found bacteria are Kock-weeks, pneumococcus and Morax-axenfeld.

Repeated monthly survey (R.M.S.)

Purpose.—To determine the nature and seasonal variation of bacterial infections of the conjunctiva and their relation to the incidence and severity of trachoma.

Five of the villages with a total population of 2,700 were kept as control. In these villages, every month, clinical examination for conjunctivitis, underlying trachoma and bacterial examination of conjunctival smears were done. Peak of associated conjunctivitis rises to the maximum height in the month of April and then again to a less greater height in the August-September period, noted for its hot and humid weather. In the cold winter months of December and January, though the incidence of clinical sub-acute and acute conjunctivitis is relatively low, the incidence of Kock-weeks positives cases was higher, thereby showing presence of large number of carriers of bacterial micro-organisms mostly Kock-weeks. Following the peaks of seasonal conjunctivitis, there is a tendency for the trachoma active cases to increase in number and severity.

In three of these 5 R.M.S. villages weekly quantitative (Grill method) and qualitative estimation of fly population was carried out to establish, if any, the relation of increased fly density to the peaks of seasonal conjunctivitis. These investigations have revealed that there is a definite relation, i.e. the conjunctivitis and the fly density rising in the same period of the year. Also, during these peaks the proportion of musca sorbens increases in relation to musca Nebulo.

Pilot treatment.—2,650 active cases of trachoma in 22 villages were given short term intermittent treatment with 1 per cent aureomycin eye ointment (two applications a day for three days, repeated every fortnight.

Total number of such cycles given=7). They were re-examined soon after the cessation of treatment and will be re-examined in 1958 after about six months of follow-up.

4,000 students in 5 city and 37 rural primary schools were examined and 725 of the active cases found were given continuous treatment with aureomycin 1 per cent eye ointment applied twice daily for 60 working days. Re-examination immediately after the treatment has been done but the long term follow up will continue in first quarter 1958.

Seminar on communicable eye diseases (11th. to 30th November 1957).—The working of the first year Trachoma Pilot Project came to an end after the seminar on communicable eye diseases held for 3 weeks at Aligarh. The objectives of the seminar were :-

1. To help young ophthalmologists to gain further experience in the clinical and laboratory diagnosis of trachoma, with particular reference to the epidemiology of the disease.
2. To indoctrinate them into the methodology of field investigations.
3. To give them more experience in trachoma control measures.

Twenty-five delegates from the States of India (with the exception of Kashmir and Jammu, Mysore, Kerala) and from Burma and Thailand attended this seminar, where various aspects of trachoma field, laboratory and clinical were exhaustively discussed.

The detailed analysis of the first year report and the proceedings of the seminar are being compiled.

243. Encephalitis Research Unit under the Principal, Lady Hardinge Medical College, New Delhi.

Fifty-two cases of clinical virus encephalitis and nine cases of encephalopathies, closely resembling encephalitis were studied in this series which comprised of eight adults and fifty-three children. It was possible this year to carry out detailed biochemical studies owing to better organization. Virus cultures were carried out on the specimens at the Virus Research Laboratories, Pasteur Institute, Coonoor.

I. CLINICAL FEATURES

(a) *Children*.—The symptomatology did not differ markedly from that of last year. There were, however, some new features, such as petit-mal attacks and peripheral neuritis in this year's series.

(b) *Adults*.—The clinical features did not differ significantly from those noted last year.

II. LABORATORY DATA

There was a polymorpho-nuclear leucocytosis in the blood in about half the cases. The cerebro-spinal fluid showed no uniform change.

III. VIRUS STUDIES

From a preliminary report from Pasteur Institute, Coonoor, it is learnt that virus has been cultured from the faeces of two of these patients.

IV. PAPER-CHROMATOGRAPHY

Paper-chromatography has been started on the cerebro-spinal fluid with a view to determine the amino acid and sugar patterns.

Reports from other countries suggest that this form of investigation may yield results which might help to distinguish between encephalitis and encephalopathies. For instance, in demyelinating diseases there is a variation in the galactose content of the C.S.F. In experimental encephalitis there is an increase in ribose in the C.S.F.

244. Inquiry on "chemotherapy of virus infections" by Dr. V.N. Krishnamurthy, at the Vaccin Institute, Bangalore.

The following compounds have been tested for the antiviral activity using influenza virus (PR₈) in eggs and mice :-

1. Resacetophenone oxime.
2. 4-Methylesculetin
3. 2-Amino-5-methylbenzothiazole
4. 4-Aminothiouracil
5. Guttiferin
6. 2-Aminothiotriazole
7. Desoxyalliin

The following compounds are being tested.

1. N¹-isobutyl-N³-benzyl thiourea
2. N¹-Allyl-N³ (P-acetyl aminophenyl) thiourea
3. 2-Amino-4-phenyl thiazole
4. 2-Thiobenzimidazole
5. Thiobiazole disulphhydrate
6. Dibenzyl and Diallyl derivatives of (5)
7. 2-Benzyl-4-allyl thio semicarbazide
8. 3-Methyl-4-Benzylidene-5-Isoxazolone
9. Antibiotic principles of *Garcinia morella*.

A brief outline of the method of testing :

(a) *On eggs.*—The drugs are injected in different concentrations to eleven day old eggs and the highest concentration not lethal to the embryo is found out.

2. The eleven-day eggs are divided into four batches.

To the first batch, the drug is introduced in the highest concentration which does not kill the embryo and half an hour later the virus namely PR₈ (100 HU) 0.1 c.c. is introduced.

To the second batch, virus PR₈ (100 HU) 0.1 c.c. is introduced allantioically and half an hour later the highest concentration of the drug is introduced per yolk sac, with the third batch serving as drug control, the fourth batch serving as virus control and each batch containing 6 eggs.

These are incubated at a temperature of 35°C. They are candled after 24 hours and 42 hours to see if there is any mortality. After 42 hours the live eggs are chilled for about 5 to 6 hours and later the allantoic fluid from each egg is pipetted into individual tubes and the virus content is titrated by hæmagglutination technique.

Compound 6 (2-amino-thio-triazole) has shown some antiviral activity in combination of drug *plus* virus, i.e., drug first and half an hour later the virus.

(b) *On mice.*—The mice are infected intranasally with influenza A strain (PR₈) and the drug is then administered intraperitoneally, there being virus and drug controls. Both the mortality and the average survival time are found out.

Two drugs, namely, resacetophenone oxime, a synthetic metal chelating compound, and guttiferin, a naturally occurring plant antibiotic have shown significant inhibitory action on the virus when the drug is administered first and followed half an hour later by the virus.

245. Study on chemotherapy of virus infections under Dr. P.L. Narasimha Rao at the Indian Institute of Science, Bangalore.

About sixteen compounds with : (i) the NCS groupings and (ii) the metal chelating orthodihydroxy, β -keto alcohol and hydroxyoximino groups have been prepared for preliminary screening for their activity against vaccinia and influenza viral infections in eggs and mice. To-date nine compounds have been tested against influenza in chick embryos and in mice. Of these, Guttiferin, a naturally occurring plant antibiotic (from *Garcinia morella*) which is capable of forming a complex with copper and the chelate forming synthetic compound. Resacetophenone oxime are found to be significantly active against influenza infection in mice. These along with other compounds are under further investigation.

246. Inquiry on the isolation of virus from tropical diseases of viral origin under Dr. K.N. Neogy at the School of Tropical Medicine, Calcutta.

With a view to isolate virus, a fever case from whom no etiological agent could be detected, was taken for investigation. Stool sample, throat washing and blood were collected for study and after suitable preparation were injected into mice for detection of virus or rickettesia. Stool sample, injected intracerebrally in suckling mice, produced sickness in earlier passages; but in subsequent passages as no sickness was detected presence of any possible virus could not be reached at.

Investigation was made as to the cause of high mortality amongst smallpox cases during last epidemic in Calcutta. Virulence of the strain of virus from individual case and the presence of neutralizing antibody in the patient's serum against particular strain of virus was investigated. Out of 136 cases studied, 7 experiments were performed with mice, 9 with eggs and the rest with rabbits. In the earlier part of the epidemic the strain seemed to possess higher degree of virulence. In small group of persons (31 out 136 cases) presence of sufficient neutralizing antibody in the sera was lacking. These were early cases and cases with no history of primary vaccination.

Attempts are being made to isolate influenza virus from 30 cases of the influenza during recent epidemic. Isolation by intranasal instillation in young mice from 9 cases and in hamsters from one case did not prove to be successful. The main experiment is being carried out with fertile eggs by allantoic inoculation. Eleventh passage in one material has been reached. Definite isolation has not yet been made.

247. Studies on mutation and re-combination of Indian strains of influenza virus under Dr. I. G. K. Menon at the Pasteur Institute, Coonoor.

The original intention was to utilize the strains isolated in India in previous years for the studies. However, the onset of the influenza pandemic and the isolation of the new Asian strains of virus made it necessary to alter the scheme considerably. The marked differences of the new strains from all previous ones made it essential to include them in the study. Attention was, therefore, concentrated on isolating as many strains as possible from different areas in the country as well as from cases presenting various degrees of severity and different types of complications, such as pneumonia, encephalitis, jaundice, etc. Thirty-three strains have been isolated from the present pandemic since May and include some from cases of varying severity and complications. Efforts are also being made to obtain isolations from fatal cases.

The strains isolated till now have been investigated on some points, such as their growth characteristics, ratio of hæmagglutination titres to infectivity, ability to get adsorbed on various surfaces and to elute therefrom sensitivity to non-specific inhibitors as well as specific antibodies in

various sera and reactivity to chemical agents, such as ether, iodine and the metal chelating agents typified by EDTA. It is intended to establish some of their properties as marker characteristics after which the effects of mutagenic agents, such as colchicine, formaldehyde and other substances can be found on these markers. Significant differences have already been found between antigenically similar strains and they can be utilized for re-combination experiments.

248. Inquiry on the incidence of influenza in Calcutta, under Dr. D.N. Sengupta at R.G. Kar Medical College, Calcutta.

The study of recent epidemic of influenza in Calcutta has been made both from clinical and laboratory side.

Clinically, the disease appeared to be mild and no mortality was recorded in the series studied. The usual symptoms of the patients were, fever of varying degrees, mostly between 103° and 104° F. (oral), back-ache, cough which was mostly non-productive and sneezing at the onset. Few patients had complications, such as bronchitis, diarrhoea, etc. The duration of illness were in most cases between 6 and 7 days.

Blood examination showed in most cases, total white cell count and differential count within normal range. Culture of the throat swab usually yielded *Streptococcus viridans*, and *Neisseria catarrhalis*. Isolation of the virus was attempted from 13 clinical cases, and except one other did not prove successful. The presence of *Ps. pyocyaneus* in throat gargle escaped penicilline-streptomycine treatment and which may be a cause of the death of the embryo in some of the cases.

Demonstration of antibody :—34 paired sera were tested for the presence of influenzal antibody against PR 8, Liverpool, Govindaraj, and PAR, (the new Singapore strain). Most of the cases showed a rising titre of antibody (4 folds and more) against PAR suggesting infection with the new strain isolated in Singapore. Serum from patients having a past history of influenza where no acute sample could be obtained was also studied. Such serum showed the presence of antibody against PAR in a significant titre. Similarly, samples of acute sera of persons whose convalescent sample were not available, were also studied, which failed to show any significant titre of antibody. It may be suggested that determination of antibody in only convalescent cases, may also be helpful in eliciting past infection, provided an idea is available as regards concentration of antibody in acute samples even if paired sample are not available. The demonstration of antibody was made by hæmagglutination inhibition technique.

249. Respiratory and Intestinal Viruses Unit under the Director, Pasteur Institute, Coonoor.

TISSUE CULTURE METHODS.

Investigations were undertaken to standardize the techniques of tissue culture for growing freshly isolated monkey kidney and human

amnion cells and the propagation of standard Hela and intestinal cells. The methods have been standardized and monkey kidney and human amnion cells are being prepared as and when required. The HeLa cells and intestinal cells kindly made available by Dr. (Mrs.) Ranadive are being maintained. An interesting observation resulting from these studies is that sheep serum could be used in place of calf serum in cultures of monkey kidney and also in place of human serum in cultures of human amnion cells. Sheep serum could also be used in the maintenance medium for human amnion and HeLa cells.

RESPIRATORY VIRUSES.

Investigations are in progress to study the incidence of adeno-viruses. It is now recognized that these group of viruses are associated with pharyngo-conjunctival fever, kerato-conjunctivitis, lymphadenitis, non-streptococcal pharyngitis, febrile catarrh and primary atypical pneumonia.

One problem which is being studied is the occurrence of other respiratory viruses during the epidemic of influenza. A large number of throat gargles have been collected from cases suspected to be suffering from influenza during the recent epidemic. Fourteen specimens were inoculated on 6-day old HeLa cell cultures in 0.2 c.c. amounts; 0.8 c.c. of a maintenance medium consisting of Eagle's Basal Medium (75 per cent), tryptose broth (20 per cent) and sheep serum (5 per cent), was added. The tubes were incubated and observed for 10 days. Any cytopathogenic agent detected was passaged into fresh HeLa cell cultures. Three viral agents have been isolated so far and their nature is being studied.

Another investigation which is in progress is the study of the incidence of adeno virus infections among military recruits. It is well known that types 3, 4 and 7 account for the majority of respiratory illnesses among newly recruited soldiers while the infections are rare among seasoned soldiers. Complement-fixing antigens are under preparation and arrangements have been made for this study.

INTESTINAL VIRUSES.

During recent years considerable attention has been paid to this group of viruses which include the poliomyelitis, Coxsackie and the ECHO group. These are associated with clinical manifestations ranging in severity from a mild febrile illness to aseptic meningitis. An investigation has been in progress to determine the incidence of these infections in our area.

Cases of encephalitis and aseptic meningitis are being reported from various parts of the country. Apart from the polio viruses the other agents generally responsible for these syndromes are : Coxsackie A9 and B1 to 5 ; ECHO type 6 and presumably ECHO types 2 and 7. All these agents produce cytopathogenic changes in tissue culture and can therefore be identified.

The main investigation which is in progress is to study the ætiology of cases of encephalitis which are occurring in Delhi as well as those reported locally. The specimens from Delhi cases are kindly being made available by Dr. (Miss) Padmavati and Dr. (Mrs.) Bhatia. So far, 290 specimens have been collected. They are from cases of suspected enteric viral infections and their household contacts and from cases of encephalitis met with in Delhi and locally. Sixty-one specimens (42 faeces, 7 throat swabs and 12 cerebro-spinal fluids) have been inoculated into monkey renal epithelial cells. Three viruses have been isolated so far from faecal samples, one from a case of suspected paralytic poliomyelitis and two from cases of encephalitis occurring in Delhi. These viruses have been re-isolated in HeLa cell cultures also. Work in connection with the identification of the viruses and the preparation of standard virus pools, immune sera, etc., for serological tests is in progress.

Steps are being taken to prepare specific immune sera against standard strains of polio, Coxsackie and ECHO group of viruses.

IV. BLOOD GROUP REFERENCE CENTRE, INDIAN CANCER RESEARCH CENTRE, BOMBAY.

The blood group research has made two important contributions to clinical medicine, namely simply and almost safe blood transfusion and iso-immunization of mothers resulting in the delivery of babies with erythroblastosis foetalis. During the last two decades, the blood group techniques have advanced to such a stage that almost all the transfusion reactions due to blood group incompatibility could be avoided. This has greatly reduced the incidence of hæmolytic transfusion reactions. Besides the careful study of transfusion reactions, erythroblastosis foetalis has revealed many newer blood group antigens and antibodies.

With the expansion of the medical facilities in India, blood transfusion services will be rapidly developing all over the country. It is, therefore, necessary that some facilities be available in the country for training such personnel as well as an arrangement be made for the availability of standard reagents.

Another service which requires urgent attention on national scale, is the testing of pregnant woman for the detection of immunization due to Rh and other blood group antigens. Arrangements should, therefore, be made for the availability of standard anti-Rh₀ (anti-D) sera to the maternity clinics.

In order to meet the requirements mentioned above. Blood Group Reference Centre was established to (i) prepare and distribute standard anti-A, anti-B and anti-Rh₀ (anti-D) as well as Coombs' reagents, (ii) training of personnel in blood bank organisation and blood group serology, and (iii) assisting the transfusion services and antenatal clinics in difficult cases of crossmatching, transfusion reactions and isoimmunization.

The work of the Blood Group Reference Centre was started in February, 1957, at four institutions in Bombay.

Preparation and supply of the reagents.—The work of the Centre was started with anti-Rh₀ sera and Coombs' reagents prepared and preserved by Human Variation Unit, Indian Cancer Research Centre. Due to unexpected difficulties in getting the Rh sera from abroad many inquiries started coming to the Centre for the supply of Rh sera and Coombs' reagents from hospitals and research workers. Blood Group Reference Centre was able to assist these agencies to a limited extent from the stocks of the Human Variation Unit.

Difficulties of getting large quantities of blood from immunized pregnant women having Rh antibody led us to adopt the method of artificial immunization of Rh negative donors. Our initial experiments in this direction were successful as two of the eight donors who were used for this purpose developed sufficiently potent Rh antibody. These two donors were bled and about 400 c.c. anti-Rh₀ (anti-D) serum

is now available for the routine work of the Centre. About 50 c.c. of anti-Rh serum was obtained from the immunized pregnant mothers delivered at the Wadia Maternity Hospital.

So far, the Coombs' reagent prepared and stocked by Human Variation Unit was supplied to various institutions. Our subsequent experiments for the production of Coombs' reagent were also successful. Two rabbits were immunised with human-globulin by sodium alginate method. Both the rabbits responded and a total of about 70 c.c. of blood from each rabbit was collected. Total of 65 c.c. of undiluted absorbed serum is now ready for use in routine work.

Rh testing of pregnant women at Wadia Maternity Hospital.—All the available cord blood of the infants delivered in the hospital and the blood of their mothers were investigated for ABO and Rh blood groups. The cord bloods were further investigated by Coombs' reagent. Out of 1,391 cases studied so far, there were 61 (4.4 per cent) Rh negative women of whom 4 contained anti-Rh (D) in their serum with varying titre. Coombs' test was positive in two cases which resulted in several erythroblastosis and death of the infants. Coombs' test was negative in two other cases and the infants were normal. 232 cases with history of abortions, still-births or babies developing jaundice in earlier pregnancies were referred from the antenatal clinic of the hospital. There were 16 Rh negative women of whom as many as 8 contained anti-Rh in their serum. 38 cases of abortions and still-births were investigated, where two more cases with anti-D were found. In two out of these 14 cases with anti-D, titre was more than 1:64 and their sera could be used as diagnostic reagent. There were three cases of suspected anti-A immunisation.

Transfusion reaction.—At the K.E.M. Hospital, 408 patients were given blood transfusion of whom 28 patients had non-hæmolytic reaction shown by high temperature with rigors and 6 cases with mild reaction (8.3 per cent). No case of late reaction was noticed. None of the 34 cases showed the presence of any antibodies as evidenced by negative indirect Coombs' test.

Collection of the blood from donors was generally done in glucose citrate solution at the K.E.M. Hospital, Blood Bank. It is now being changed to ACD mixture. Cross-matching technique is also modified to a certain extent to include the test for incomplete antibodies.

Low's papain-cysteine method which was standardized at the Human Variation Unit is under study and if it proves satisfactory for routine cross matching this will be of use to detect practically all the types of incomplete antibodies except the Duffy blood group.

Biochemical studies.—Attempts were made to isolate the blood group substances from various biological materials. These group substances were required for absorbing anti-A and anti-B from Rh sera obtained from pregnant immunized women. First attempt to prepare them was made from saliva of secretors of A and B group substances. Little quantities of both these substances are now prepared. Each substance

was found to be about 30 times more active than the original saliva. AB substance is also under preparation from AB group ovarian cyst fluid.

Other biochemical aspect of the blood which was taken for study was the preparations of plasma fractions. A pilot experiment for separation of the different plasma proteins by Cohn's alcohol extraction gave small quantities of fractions, such as fibrinogen, α , β and γ globulins, prothrombin and albumin. Attempts are being made to study the purity of these fractions by electrophoresis.

Activities of Blood Group Reference Centre will be expanded by introducing two other hospitals in Bombay for blood group work.

To enure a regular supply of anti-Rh sera, more donors will be immunized. It is hoped that Blood Group Reference Centre will be able to supply other institutions a limited quantity of these sera in a short time. Coombs' reagent will also be prepared in adequate quantities.

V. INDIAN JOURNAL OF MEDICAL RESEARCH

The Indian Journal of Medical Research is published since 1913 under the aegis of the Indian Council of Medical Research (*formerly* Indian Research Fund Association), and serves as the principal organ for dissemination of scientific and medical research work carried out in India under the Council and other research institutions. Papers based only on original research are accepted for publications. The Editorial Board of the Journal consists of the leading authorities on tropical medicine in India.

The subscription list of the journal is swelling every year. From 300 subscribers last year, it has gone up to 342 this year. There are about 180 Indian and foreign periodicals of different national and international organizations on its Exchange Mailing List, and still requests for exchange relations from different countries are being received. The exchange is granted on the basis of the merit of the journal offered to the local research workers.

The journal has been appearing quarterly uptil now, and each number contains on an average 200 pages and is well-illustrated. *Memoirs* and *Supplementary Numbers* are also published as a supplementary series and are distributed, as and when published, to all on the Mailing Lists.

The journal will be published bimonthly commencing with the January 1958 number. The rate of subscription per volume (6 numbers) has been raised from Rs. 20.00 to Rs. 25.00 (including postage, *Special Supplements* and *Memoirs*). The cost of a single copy of each issue of the journal will be Rs. 5.00 (excluding postage).

VI. INDIAN JOURNAL OF MALARIOLOGY

Indian Journal of Malariology, Vol. X, No. 4, 1956.

The December 1956 issue, comprising 92 pages of printed and tabular matter, was published during the year under report.

The March, the June and the September 1956 issues, publication whereof was reported last year, contained 80, 86 and 106 pages, respectively. Thus Vol. X of the Journal comprised 364 pages of printed and tabular matter.

Besides contributors in India, the Journal continues to enjoy the confidence of research workers in foreign countries as well. Of the 33 papers published in Vol. X, 3 were submitted by workers from Nepal, Burma and Algeria.

Indian Journal of Malariology, Vol. XI, 1957.

The March and the June 1957 issues comprising 126 and 122 pages, respectively, have been published.

The September 1957 issue is in the press, and the December 1957 issue is in the process of being sent to press.

DISTRIBUTION.

Requests from institutions in India and abroad are received regularly for inclusion of their names in the Exchange Mailing List, and where the literature published by the parties, is considered sufficiently useful, such requests are invariably entertained. The sub-jointed table shows the distribution of the Journal.

SUBSCRIBERS.

Foreign countries	...	...	70	
In India	...	...	98	168

Exchange mailing list	...	...	...	114
Free mailing list	...	...	...	112
Voucher copies to advertisers, specimens, propaganda etc.	...	...	...	25
Malaria Institute of India		...	...	61
Balance held by the publishers for sale			...	120

				600

**VII. (a) MICROFILM AND PHOTOCOPY SERVICE UNIT
AT THE CENTRAL RESEARCH INSTITUTE,
KASALI.**

REPORT OF WORK DONE DURING THE PERIOD
OCTOBER, 1956 TO SEPTEMBER, 1957.

During the period under review in all 67 requests for Microfilms and Photostat copies were received out of which only 53 were complied with. Seven requests for which the publications were not available at Kasali, were transferred to the Bombay Unit and INSDOC at New Delhi.

1188 pages of microfilms and 524 pages of photostat copies were supplied to medical institutions and research workers in India and abroad.

The following table compares the current figures with the amount of work done during the period October, 1955 to September, 1956.

	<i>1955-56</i>	<i>1956-57</i>
Microfilms	1192 pages	1188 pages
Photostat copies	721 pages	524 pages

The supply of microfilms has almost remained steady, the Insdoc being our major indentors as usual, while the supply of Photostat copies has slightly decreased.

VII. (b) MICROFILM AND PHOTOCOPY SERVICE UNIT AT THE TATA MEMORIAL HOSPITAL, BOMBAY.

During the period under report 214 requests for microfilms and photocopies were attended to whereas 24 requests could not be complied with because of the non-availability of the journals in Bombay. 4,327 pages were microfilmed and 1,526 pages were photocopied for various medical and scientific institutions and research workers listed below. The following table gives a comparative statement of the documentation work done during the year under report and the previous year.

	Oct. 1955	Sept. — 1956	Oct. 1956	Sept. — 1957
Pages Microfilmed		2,975		4,327
Pages Photocopied		1,227		1,526

The rates for the supply of Microfilms and Photocopies are :—

MICROFILMS :

For the first ten pages	...	...	Rs. 3.00
For the subsequent ten pages or any portion thereof			Rs. 1.50

PHOTOCOPY :

Size upto	11" × 16"	...	...	Rs. 0.50
„ „	17" × 11"	...	...	Rs. 0.75
„ „	23" × 17"	...	...	Rs. 1.87

VIII. PAPERS PUBLISHED BY WORKERS OF THE INDIAN COUNCIL OF MEDICAL RESEARCH DURING THE YEAR 1957.

(a) *In the Indian Journal of Medical Research*

1. BHAGAVAN, N.V., NIMBKAR, Y.S., & RAO, S.S. Fractionation of the Soluble Antigens of *Pasteurella pestis*.
2. BANERJEE, S., BHADURI, J.N., & SARKAR, A.K. Studies on Glucose Tolerance Test in Cholera.
3. SEN, N.N., KUMAR, S., & MANGALIK, V.S. Rôle of Spleen in the Maintenance of Peripheral Leucocytes. Part III. Effect of Spleen Extract on Peripheral Leucocyte Number (*with 4 Graphs in text*).
4. MUKHERJEE, S.K., DE, U.N., & MUKERJI, B. Studies in Experimental Diabetes. Part V. Effect of Treatment with Vitamin E and Vitamin K, and Their Combination with Œstrogen on the Course of Alloxan Diabetes in Rats (*with 1 Plate*).
5. BANERJEE, S., & SEN, R. A Nomogram for Calculating the Surface Area of the Body of Indian (*with 1 Text-figure*).
6. PAIN, S.K., & BANERJEE, S. Studies on the Nitrogenous Constituents of Urine in Normal Subjects and in Patients Suffering from Cirrhosis of Liver, Subacute Nephritis and Hypertension.
7. DAS GUPTA, S.K., & BAWA, Y.S. Studies on Urinary Excretion of 17-ketosteroids and on Liver Function Tests in Infective Hepatitis.
8. PRADHAN, S.K., & MAGAR, N.G. Biological and Chemical Estimation of Vitamin D in Shark-liver Oil.
9. BALAKRISHNAN, S., BALIGA, B.R., & RAJAGOPALAN, R. Effect of Continued Feeding of Curds on the Intestinal Flora and Biosynthesis of Thiamine in Rats (*with 2 Graphs in text*).
10. GOPALAN, C., & RAMANATHAN, K.S. Response to Water Load Tests in Induced Protein Deficiency.
11. DE, N.K., & DIKSHIT, P.K. The Alleged Rôle of Dietary Phosphorus in Utilization of Carotene (*with 2 Graphs in text*).
12. VENKATARAMAN, R., & CHARI, S.T. Amino-acid Composition of Some Fishes.
13. CHARI, S.T., & VENKATARAMAN, R. Semi-drying of Prawns and its Effects on the Amino-acid Composition.

14. NARASINGA RAO, B.S., & GOPALAN, C. Some Aspects of the Hair Changes in Kwashiorkor.
15. PAI, M.L. Influence of Cooking on the Nutritional Value of Foods. Part I. Thiamine Content of Some Cooked Foods (*with 1 Graph in text*).
16. VALANJU, N.N., & SOHONIE, K. The Studies in the Nutritive Value of Bombay Fish. Part II. Isolation, Purification, Nitrogen Distribution and the Amino-acid Make-up of Fish Proteins.
17. VALANJU, N.N., & SOHONIE, K. The Studies in the Nutritive Value of Bombay Fish. Part III. Essential Amino-acid Make-up of Proteins of Ghol and Mandeli.
18. VALANJU, N.N., & SOHONIE, K. The Studies in the Nutritive Value of Bombay Fish. Part IV. *In vitro* Digestibility of Fish Proteins (*with 2 Graphs in text*).
19. VALANJU, N.N., & SOHONIE, K. The Studies in the Nutritive Value of Bombay Fish. Part V. The Digestibility and the Biological Value of Fish Proteins (*with 1 Graph in text*).
20. INDAR JIT. The Development of the Sphincter of Oddi (*with 2 Plates and 2 Text-figures*).
21. AGARWAL, S.C. The Hæmolytic Response of Anti-erythrocytic Sera in Pulmonary Tuberculosis.
22. SRIKANTAN, T.N., AGARWALA, S.C., & SHRIVASTAVA, D.L. Studies in the Enzyme Make-up of *Pasteurella pestis*. Part III. Oxidative Metabolism of Virulent and Avirulent Strains (*with 3 Graphs in text*).
23. SAXENA, K.C., SAGAR, P., AGARWALA, S.C., & SHRIVASTAVA, D.L. Studies in the Enzyme Make-up of *Pasteurella pestis*. Part IV. Transamination Reactions in Virulent and Avirulent Strains (*with 4 Graphs in text*).
24. GANGULI, S. Neutral Red Test for Virulent *Mycobacterium tuberculosis*.
25. BANNERJI, A.K., GUPTA, N.P., & GUPTA, S.P. Sensitization of Red Cells with Extracts of Salmonella Organisms for Hæmagglutination Test.
26. MEHROTRA, R.M.L., MANGALIK, V.S., & NAYAK, N.C. An Experimental Study of the Effect of Doca on the Development of Ascites in CCL₄ Cirrhosis (*with 2 Text-figures*).
27. DAS, B.R., KURUP, P.A., & NARASIMHA RAO, P.L. Antibiotic Principle from *Moringa pterygosperma*. Part VII. Antibacterial Activity and Chemical Structure of Compounds Related to Pterygospermin.

28. DAS, B.R.,
KURUP, P.A.,
NARASIMHA RAO,
P.L., &
RAMASWAMY, A.S. Antibiotic Principle from *Moringa pterygosperma*. Part VIII. Some Pharmacological Properties and *In vivo* Action of Pterygospermin and Related Compounds (*with 3 Text-figures*).
29. GUJRAL, M. L.,
SAREEN, K.N., &
KOHLI, R.P. Evaluation of Anticonvulsant Activity of 2, 3-Disubstituted Quinazolones: A New Class of Anticonvulsant Drugs (*with 2 Graphs in text*).
30. IYER, G.Y.N. Paper-chromatographic Study of Free Amino Acids in Normal and Anæmic Erythrocytes (*with 2 Text-figures*).
31. NATH, H.P.,
GUPTA, K.K., &
IYER, P.V.K. Serum Cholesterol and Phospholipid Contents of Normal Indian Service Personnel.
32. SACHDEV, J.C. Study of the Preventive Action of the Anti-fatty Liver Fractions of Pancreas on Fatty Liver Induced by Ethionine (*with 1 Text-figure and 1 Graph in text*).
33. SENGUPTA, A.N.,
CHAKRABARTY, B.,
& KONAR, N.R. Observations on Intrapleural Pressure in Healthy Men.
34. ROY, S.N., ROY,
S.K., & KARKUN,
J.N. The Effect of Testosterone Propionate on the Adrenal Cortex. Part I. Influence of Androgens on the Pituitary Andrenal Function (*with 1 Plate*).
35. BAL KRISHNA, &
CHAKRAVARTI, R.N. Electrophoretic Fractionation of Serum Proteins in Experimental Cholesterol Atherosclerosis (*with 2 Text figures*).
36. BOSE, S.K. Significance of Multiple Pathways in Adaptation. Part II. Inhibition of Germination of *A. niger* as Induced-by Exposure to Phenylmethane Dyes.
37. RAMANATHAN, M.K.,
& GOPALAN, C. Effect of Different Cereals on Blood Sugar Levels (*with 4 Graphs in text*).
38. SESHADRI, SASTRY,
P., KRISHNAMURTHY,
S., & GANGULY J. Studies on Vitamin A Esterase, Part I. The General Properties and Possible Specificity of the Rat-liver Enzyme (*with 5 Graphs in text*).
39. VENKATESWARLU, P.,
& NARAYANARAO, D. A Convenient Method for the Estimation of Fluorine in Waters Containing Excessive Amounts of Interfering Ions.
40. RAMANATHAN, N.L.,
ROY, B.N., &
SEN GUPTA, A. Estimation of the Ionic Constituents of Human Sweat by the Electrical Conductivity Method.

41. AGGARWAL, M.L., & PATHAK, I.C. Roentgenologic Study of Epiphyseal Union in Punjabi Girls for Determination of Age.
42. AGARWAL, S.C. Serum Glycoprotein Response During Immunization. Part I. Effect of Inadequate Antigenic Stimuli (*S. typhi*).
43. BHATIA, A.L., GHAI, S.N., & DUDANI, A.T. Relationship of Serum Glycoprotein (Hexose) and Blood Sedimentation Rate in Pulmonary Tuberculosis Patients.
44. SURI, J.C. Anti-typhoid Vaccine. Part I. Choice of an Active-protection Test.
45. SRIVASTAVA, G.N., CHAKRAVARTI, R.N. & ZAIDI, S.K. Studies in Experimental Atherosclerosis. Part IV. Serum Fibrinolysin Activity in Cholesterol Atherosclerosis (*with 1 Graph in text*).
46. CHAKRAVARTI, R.N., DE, U.N., & MUKERJEE, B. Studies in Experimental Atherosclerosis. Part V. Therapeutic Effect of Ascorbic Acid and Vitamin B₁₂ in Cholesterol Atherosclerosis.
47. SAXENA, P.N., & GUPTA, G.P. Potentiating Effect of Prostigmine on Morphine-induced Analgesia (*with 2 Graphs in text*).
48. GOKHALE, S.V., & GODBOLE, S.H. Serum Lipolytic Enzyme Activity and Serum Lipid Partition in Leprosy and Tuberculosis.
49. MUKERJEE, S.K., DE, U.N., & MUKERJEE, B. Alpha Cell Activity and its Relation to Glycæmic Level and Insulin Sensitivity in Albino Rats (A Comparative Study of the Action of Nadisan and Cobalt Chloride) (*with 2 Graphs in text*).
50. ANAND, B.K., DUA, S., & CHHINA, G.S. Changes in Visceral and Metabolic Activities after Frontal and Temporal Lobe Lesions (*with 3 Text-figures*).
51. ANAND B.K., DUA, S., & CHHINA, G.S. Changes in the Affective Behaviour Produced by Lesions in the Frontal and Temporal Lobes.
52. NATH, H.K., & CHAKRAVARTI, B. Studies on Plasma Sodium and Potassium in Surgical Conditions.
53. RINDANI, T.H., & MERCHANT, R.C. Effect of Acetylcholine on Cooled Heart (*with 3 Text-figures*).
54. VENKATESWARLU, P., & NARAYANARAO, D. Estimation of Fluorine in Biological Material.
55. VENKATESWARLU, P., & NARAYANARAO, D. An Evaluation of Vitamin C Therapy in Fluorine Intoxication.

56. VENKATESWARLU, P., & NARAYANARAO, D. Distribution of Fluorine in the Tissues of Rats.
57. KRISHNAMURTHY, S., SESHADRI SASTRY, P., & GANGULY, J. Studies on Vitamin A Esterase. Part II. The Hydrolysis of Natural Vitamin A Ester within the Living Animal.
58. GANAPATHY, SEETHA N., CHITRE, R.G., & GOKHALE, S.K. The effect of the Protein of Italian Millet (*Setaria italica*) on Nitrogen Retention in Albino Rats.
59. SATHE, V.R., TALAGERI, V.R., KHANOLKAR, V.R., & PANSE, T.B. Studies on B-vitamins and Estrogens in Oral Cancer.
60. BANERJEE, S., & BISWAS, D.K. Studies on the Nutritive Value of the Cooked Diet Consumed by Students of the Eden Hindu Hostel, Calcutta.
61. GOPALAN, C., & PADMAVATHI, S. Electrocardiographic Studies on Normal and Malnourished Monkeys (*Macacus-mullatus*) with 3 Text-figures)
62. JAYALAKSHMI, V.T., RAMANATHAN, M.L., & GOPALAN, C. Some Observations on the Effects of Treatment of Cases of Kwashiorkor.
63. JOSHI, J.G., DIKSHIT, P.K., & PATWARDHAN, V.N. Mode of Action of Vitamin D. Citrogenase and Citric Acid in Dogs in Induced Rickets (with 1 Graph in text).
64. RAMA SASTRI, B.V., & INDRAVATI, D. Investigations on the Thiamine Content of Human Milk in Andhra.
65. CHITRE, R.G., & RAUT, V.S. The Rôle of the Alkali-labile Bound Form of Niacin during the Growth of Paddy.
66. DESAI, D.B., & CHITRE, R.G. Niacin Biosynthesis in Germinating Cereals and Pulses. Part II. Further Observations on the Effect of Tryptophane on Alkali-labile Precursor of Niacin during Germination in Cereals.
67. ANNOUNCEMENT re Colonel Amir Chand Trust Prizes for Medical Research.
68. SRIKANTAN, T.N., AGARWALA, S.C., & SHRIVASTAVA, D.L. Studies in the Enzyme Make-up of *Pasteurella pestis*. Part V. Dehydrogenase Activity of Virulent and Avirulent Strains (with 5 Graphs in text).

69. AGARWAL, S.C. Serum Glycoprotein Response During immunization. Part II. Effect of Adequate Antigenic Stimuli (*S. typhi*) (*with 6 Graphs in text*).
70. KUMAR, S.,
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SEN, N.N. Rôle of Spleen in the Maintenance of Circulating Leukocytes. Part IV. Effect of Splenectomy on the Myeloid Elements of Bone Marrow in Albino Rats (*with 4 Graphs in text and 1 Plate*).
71. Dutta, N.K., &
THADANI, K.C. Fibrin (Horse) Hydrolysate for Parenteral Administration (*with 1 Graph in text*).
72. ANAND, B.K.,
MALKANI, P.K., &
DUA, S. Effect of Electrical Stimulation of the Hypothalamus on Menstrual Cycle in the Monkey (*with 2 Graphs in text*).
73. ANAND, B. K.,
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SIKAND, S. Hypothalamic Control Over Œstrous Cycle in the Rat (*with 3 Graphs in text*).
74. SEN, R.N., &
ANAND, B.K. Effect of Electrical Stimulation of the Hypothalamus on Gastric Secretory Activity and Ulceration (*with 1 Text-figure and 1 Plate*).
75. SEN, R.N., &
ANAND, B.K. Effect of Electrical Stimulation of Limbic System of Brain (Visceral Brain) on Gastric Secretory Activity and Ulceration.
76. RINDANI, T.H. Relationship of Male Sex Hormone to Stress Induced Activation of Adrenal Cortex in Rat.
77. RINDANI, T.H.
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78. BANERJEE, S., &
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D. Studies on the Rôle of Methionine in the Metabolism of Tryptophane by Rats Treated with Carbon Tetrachloride.
79. BANERJEE, S., &
BISWAS, D.K. Studies on the Nutritional Status of Students of the Eden Hindu Hostel Attached to Presidency College, Calcutta.
80. PRASAD, J.N. The Electro-retinogram of Bat and Owl (*with 2 Text-figures*).
81. BAL KRISHNA,
CHAKRAVARTI, R.N.,
& MUKERJI, B. Effect of Lipid Intake on the Serum Protein and Lipo-protein Pattern in Experimental Atherosclerosis (*with 1 Graph in text*).
82. VISHWAKARMA, P.,
& SINHA, Y.K. The Effect of Oxyphenonium, Hexamethonium and Probanthine on the Gastric Acid Secretion of Rats.

83. CHOPRA, I.C., Pharmacological Action of *Lochnera rosea* Linn.
JAMWAL, K.S., (Rattanjot)
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84. DEOLALKAR, S.T., Studies on Thiaminase from Fish. Part I.
& SOHONIE, K. Properties of Thiaminase (*with 3 Graphs in text*).
85. DEOLALKAR, S.T., Studies on Thiaminase from Fish. Part II.
& SOHONIE, K. Effect of Certain Compounds on Thiaminase
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86. GOPALAN, C., & Studies on Blood Cholesterol Levels of Healthy
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1 Graph in text).
87. JAYALAKSHMI, V.T., Studies on the Blood of Poor Indian New-Born
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88. PHANSALKAR, S.V., Nutritive Value of Vegetable Proteins. Part I.
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& PATWARDHAN, Pulses and the Supplementary Effect of the
V.N. Addition of Leafy Vegetable.
89. BALASUBRAMANIAN, Amino-acid Composition of Indian Foodstuffs.
S.C., & Part III. Threonine and Arginine Content of
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90. DAS, M.L., & A Method of Estimation of Bound Nicotinic
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91. PAI, M.L. Influence of Cooking on the Nutritional Value
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92. NARAYAN, D., & Ages of Epiphyseal Union in Long Bones of
BAJAJ, I.D. Inferior Extremity in U.P. Subjects.
93. SEKHON, G.S. The Mechanisms of Non-penetrating Injuries
to the Hollow Abdominal Viscera (*with 1 Text-*
figure).

(b) *Indian Journal of Malariology*

1. BAMI, H.L., & Studies on particle size distribution in relation
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water dispersible powders. (Vol. 11, No. 2)
2. BAMI, H.L., D.D.T. water dispersible powders: Studies
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3. BASU, P.C. Filariasis in Assam State. (Vol. 11, No. 3)

4. BHATIA, M.L., & WATTAL, B.L. Description of a hitherto unknown structure in the mouth parts of mosquitoes. (Vol. II, No. 2)
5. BHATIA, M.L., WATTAL, B.L. & KALRA, N.L. Structure of salivary glands in mosquitoes. A preliminary note. (Vol. II, No. 1)
6. BHATIA, M.L., WATTAL, B.L. & MAMMEN M.L. Preferential indoor resting habits of *A. culicifacies* Giles near Delhi. (Vol. II, No. 1)
7. DHIR, S.L. & RAHIM, A. Malaria and its control in Afghanistan (1950-1954). (Vol. II, No. 1)
8. GREWAL, M.S. On two new piroplasms from the African mongoose, *Helogate undulata rufula*. (Vol. II, No. 2)
9. GREWAL, M.S., DAVID, A. & CHOWDHARY, D.S. Preliminary report of the further studies on the (so called) natural cryptic (occult) trypanosome of the house-sparrow, *Passer domesticus* Linnæus. (Vol. II, No. 4)
10. JASWANT SINGH, ANANTHASWAMY RAO, B. & RAMAKRISHNAN, S.P. National malaria control programme in India, 1953-1956. (Vol. II, No. 4)
11. MISRA, B.G., & RAY, A.P. Detection of malaria parasites by concentration method. (Vol. II, No. 3)
12. NEOGY, B.P., & KACHROO, P. Association of *Chara zeylanica* Willd. with anopheline breeding within Damodar-Eden Canal Area of West Bengal. (Vol. II, No. 3)
13. NEOGY, B.P., KACHROO, P. & BISWAS, K. Aquatic vegetation of Bokaro Reservoir in relation to anopheline mosquito larvae and its control by application of herbicides. (Vol. II, No. 2)
14. PATEL, T.B., RAMACHANDRA RAO, T. & PARANJPEY, P.D. A pilot study on the suspension of D.D.T. spraying and the setting up of a surveillance organization in Kanara District covering the work up to October 31, 1956. (Vol. II, No. 3)
15. RAHMAN, J., SINGH, M.V. & GUJRAL, J.S. Investigation of filariasis problem in Ballia Town (Uttar Pradesh). (Vol. II, No. 2)
16. RAMACHANDRA RAO, T. & BHATIA, S.C. A note on the degree of susceptibility of *A. culicifacies* to D.D.T. in some parts of Bombay State. (Vol. II, No. 3)
17. RAMCHANDRA RAO, T. & RAJAGOPALAN, P.K. Observations on mosquitoes of Poona District, India, with special reference to their distribution, seasonal prevalence and the biology of adults. (Vol. II, No. 1)

18. RAMAKRISHNAN, S.P., SATYA PRAKASH, & CHOUDHURY, D.S. Studies on *Plasmodium berghei* Vincke and Lips, 1948. Part XXIV. Selection of a chloroquine resistant strain. (Vol. II, No. 2)
19. RAY, A.P. Studies on Nuri strain of *P. knowlesi*. Part XIV. Pathological progress in the liver. (Vol. II, No. 2)
20. RAY, A.P. Hæmatological studies in simian malaria. Part I. (Vol. II, No. 4)
21. RAY, A.P. Hæmatological studies in simian malaria Part II. (Vol. II, No. 4)
22. RAY, A.P. Hæmatological studies in simian malaria. Part III. (Vol. II, No. 4)
23. SATYA PRAKASH. Studies on *Plasmodium berghei* Vincke and Lips, 1948. Part XXV. The role of casien diet on the acquired immunity of mice infected with *P. berghei*. (Vol. II, No. 2)
24. SERGUEEV, P.G., & DEMINA, N.A. Studies on immunity to *Plasmodium berghei* infection. Part I. The transmission of acquired immunity against *P. berghei* from female rats to their Offspring. (Vol. II. No. 2)
25. SHARMA, M.I.D., & BAMI, H.L. Comparative efficacy of 50 per cent dieldrin-resin dispersible powder and 50 per cent dieldrin dispersible powder. (Vol. II, No. 2)
26. SHARMA, M.I.D., BAMI, H.L., & KRISHNAMURTHY, B.S. Studies on the rapid loss of biological efficacy of D.D.T. and dieldrin suspensions on mud surfaces against culicine mosquitoes. (Vol. II, No. 3)
27. SHARMA, M.I.D., & KRISHNAMURTHY, B.S. Studies on the susceptibility of *A. subpictus* Grassi 1899 to chlorinated hydrocarbon insecticides in a Delhi State village. (Vol. II, No. 2)
28. SHARMA, M.I.D., KRISHNAMURTHY, B.S., & SINGH, N.N. Biometric studies on susceptibility of some mosquitoes to D.D.T., B.H.C. and dieldrin, (Vol. II, No. 4)
29. SUNDARARAMAN, S. SOEROTO, R.M., & SIRAN, M. Vectors of malaria in Mid-Java. (Vol. II, No. 4)
30. VENKAT RAO, V. On the importance of limnological studies in some malaria eradication schemes. (Vol. II, No. 4)

IX. LIST OF PAPERS ON WORK DONE UNDER THE AUSPICES OF THE INDIAN COUNCIL OF MEDICAL RESEARCH PUBLISHED DURING THE YEAR 1957.

CLINICAL.

1. ANDLEIGH, H.S. Etiology of Maduromycosis in India, Mycopathologia etc. Mycologia applicata (Vol. No. 8, 138-153, 1957).
2. ANDLEIGH, H.S. *In vitro* study of antifungal activity of pentamidine and stilbamidine, Mycopathologia etc. Mycologia applicata (Vol. No. 8, 135-137, 1957).
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6. CHARI, M.V. Tropical Pulmonary Eosinophilia—Indian Year Book of Medical Sciences, 1957-58. (Current Medical Practice)
7. CHATTERJEA, J.B. Splenic puncture : Its role in heamatological disorders. Jour. Exper. Med. Science. **1** : 1-16, (1957)
8. CHATTERJEA, J.B. Haematology. Bull. Cal. Sch. Trop. Med. **5** : 108-111. (1957)
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13. CHATTERJEA, J.B., SAHA, A.K., RAY, R.N., & GHOSH, S.K. Haemoglobin E thalassaemia disease. *Ind. Jour. Med. Sci.*, **11**, 553, (1957).
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17. CHAUDHURI, R.N., RAI CHAUDHURI, M.N., SEN, P., & BASU, S.P. Studies on tropical eosinophilia II. Treatment with diethyl carbamazine *Ibid.*, Vol. **4**, 150, (1956).
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23. KAMAT, V.B., PANSE, T.B., & KHANOLKAR, V.R. Chemistry of Human Smegma in relation to cancer (Ready for publication).
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2. CHATTERJEE, K.R., & MUKERJEE, N. Experimental Transmission of Myco-bacterium leprae Murium (Stefansky's bacillus) to a selected hybrid black mouse. *Bull. Cal. Sch. Trop. Med.* Vol. V, pp. 3-7, 1957.
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